Evolution d'un déterminisme du sexe atypique chez un mammifère : causes et conséquences

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Evolution d'un système de déterminisme du sexe atypique chez un mammifère

Causes et conséquences

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EVOLUTION D'UN SYSTEME DE DETERMINISME DU SEXE ATYPIQUE CHEZ UN MAMMIFERE, CAUSES ET CONSÉQUENCES.

Le système de déterminisme du sexe des mammifères thériens (XX/XY) est ancien et conservé : toute déviation mène généralement à la stérilité. Cependant, quelques espèces dérogent à la règle. C'est le cas de la souris naine africaine *Mus minutoides*, qui possède un système de déterminisme polygénique où les mâles sont XY, et les femelles XX, XX* ou X*Y (l'astérisque désigne une mutation sur le X, féminisant les embryons X*Y, et apparue il y a presque 1 million d'années). L'évolution d'un tel système est un paradoxe : les femelles X*Y sont censées faire face à des coûts reproductifs importants (perte d'embryons YY, problèmes de méiose…), qui devraient empêcher le maintien de la mutation. Afin de mieux comprendre l'évolution de ce système, nous avons dans un premier temps cherché à identifier les mécanismes évolutifs impliqués dans l'émergence et le maintien du X*. La combinaison d'une approche empirique et d'une étude théorique basée sur des modèles de génétique des populations a permis de mettre en évidence que deux facteurs participent au maintien du X*: un meilleur succès reproducteur des femelles X*Y et la présence de distorteurs de transmission des chromosomes sexuels mâles (leur Y est transmis majoritairement dans les croisements avec des femelles XX et XX* et leur X avec des femelles X*Y). Ce second facteur est certainement à l'origine de l'émergence de ce système. Nous avons ensuite analysé les conséquences de l'évolution de ce système atypique avec trois chromosomes sexuels d'abord sur le phénotype : alors que les trois types de femelles sont indistinguables morphologiquement, les femelles X*Y présentent un comportement masculinisé (elles sont plus agressives et moins anxieuses), puis sur l'évolution de la séquence et de la structure du X et du X* (basé sur des données de séquençage NGS), mettant en évidence que ces chromosomes ont commencé à diverger. Dans l'ensemble, cette étude permet de mieux comprendre les contraintes agissant sur les systèmes de déterminisme du sexe anciens, et les conditions exceptionnelles pouvant réduire ces contraintes permettant ainsi l'évolution d'un nouveau système de déterminisme du sexe. Elle améliore aussi la compréhension de l'impact du complément en chromosomes sexuels sur le phénotype et renseigne sur les forces évolutives agissant sur les chromosomes sexuels dans ce type de système de déterminisme polygénique.

Mots-clés : souris naine africaine, femelles XY, chromosomes sexuels, sex-ratio, modélisation, comportement, génomique

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EVOLUTION OF AN UNUSUAL SEX DETERMINATION SYSTEM IN A MAMMAL, CAUSES AND CONSEQUENCES.

Therian mammals have an extremely conserved XX/XY sex determination system. Their highly differentiated and specialised sex chromosomes are thought to prevent any modification; however, a dozen species harbour unconventional systems. In the African pygmy mouse *Mus minutoides*, all males are XY, and there are three types of females: the usual XX but also XX* and X*Y ones (the asterisk designates a sex reversal mutation on the X chromosome, which evolved almost 1 million years ago). The evolution of such a system is a paradox, as X*Y females are expected to face high reproductive costs (loss of YY embryos, meiotic problems…), which should prevent the maintenance of the mutation. To better understand the evolution of this curious system, we first tried to identify the evolutionary mechanisms involved in the emergence and maintenance of the X*. The combination of empirical data and a theoretical approach based on population genetics models showed that two mechanisms participate in the maintenance of the system: the greater breeding success of X*Y females and the presence of sex chromosome transmission distorters (males transmit their Y more often in crosses with XX or XX* females and their X in crosses with X*Y females), the second mechanism likely being the trigger for the initial spread of the feminising chromosome. We then investigated the consequences of the evolution of this unusual system with three sex chromosomes. First on the phenotype, revealing that despite X*Y females have typical female anatomy and morphology, they resemble males on certain aspects of behaviour: they are more aggressive and less anxious than XX and XX* females. Then on the sequence and structural evolution of the X and X* (based on NGS data), showing that the two chromosomes have started diverging. Altogether, these results shed light on the constraints acting on sex determination systems with highly heteromorphic sex chromosomes and show that rare conditions can loosen these constraints. They also provide valuable insight into the impact of sex chromosome complement on phenotype, and inform on the evolutionary forces acting on sex chromosomes in that kind of polygenic sex determination system.

Key-words: African pygmy mouse, XY females, sex chromosomes, sex-ratio, mathematical modelling, behaviour, genomics

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Introduction

La reproduction sexuée est presque universelle chez les eucaryotes. Ce type de reproduction est caractérisé par deux processus : la méiose et la syngamie, en d'autres termes la fusion de deux gamètes. Alors que chez beaucoup d'organismes unicellulaires, tous les gamètes produits sont identiques (on parle d'isogamie), on retrouve chez la plupart des eucaryotes multicellulaires deux types de gamètes (anisogamie). Cette dichotomie est cruciale car c'est ce qui définit fondamentalement les sexes: les femelles produisent de gros gamètes immobiles en petit nombre et les mâles de petits gamètes mobiles en grand nombre. L'anisogamie, qui a évolué à maintes reprises indépendamment au sein de différentes lignées, conduit à la divergence des stratégies d'allocation des ressources caractéristiques des mâles et des femelles, et bien souvent, à leur différenciation. Chez les organismes à sexes séparés, cette différenciation sexuelle requiert l'existence de facteurs qui déterminent si un individu en développement va produire de gros ou de petits gamètes, et donc devenir une femelle ou un mâle. Le processus clé initiant la différentiation sexuelle est ce qu'on appelle **le déterminisme du sexe**. On pourrait s'attendre à ce qu'un processus aussi fondamental d'un point de vue biologique et évolutif soit extrêmement conservé, mais ce n'est pas le cas, les mécanismes impliqués sont au contraire remarquablement divers et très dynamiques au sein de nombreux taxons.

Le déterminisme du sexe fascine les biologistes depuis de nombreuses années. Son étude tient une place importante en médecine humaine et en biologie du développement, du fait du nombre important de pathologies impliquant des troubles du déterminisme du sexe chez l'Homme. C'est également un sujet en plein essor en biologie évolutive, notamment grâce au développement fulgurant des outils de séquençage à haut débit qui permettent de faciliter l'identification des chromosomes sexuels chez les organismes non-modèles, et aussi de comprendre les conséquences évolutives de la présence de tel ou tel système de déterminisme sur le génome.

Au cours de ma thèse, je me suis intéressé à l'évolution du déterminisme du sexe d'un curieux mammifère, **la souris naine africaine**, dont on peut dire que les caractéristiques génomiques sont hors normes. Cette espèce possède un mode de déterminisme sexuel remarquable : certaines femelles ont un complément en chromosomes sexuels XY, habituellement propre aux mâles. Sa proximité phylogénétique avec la souris de laboratoire, organisme modèle par excellence, fait de la souris naine Africaine un modèle prometteur pour mieux comprendre le déterminisme du sexe des mammifères et donc de

l'Homme, et plus généralement étudier l'évolution du déterminisme du sexe. C'est ce dernier point qui a motivé ces trois années de recherche.

En introduction, je passe en revue quelques concepts nécessaires à la compréhension de mon exposé. En me focalisant sur les métazoaires et bien souvent sur les vertébrés, j'y parle entre autres de la remarquable diversité des modes de déterminisme sexuel et des hypothèses actuelles pour expliquer les transitions d'un système de déterminisme à un autre. Ensuite j'aborde de manière assez générale le déterminisme du sexe chez les mammifères, avant de présenter le modèle biologique et la problématique : quelles sont les causes et conséquences évolutives de la modification du déterminisme du sexe chez la souris naine Africaine ?

1. Le déterminisme du sexe

A. Modes de déterminisme du sexe

Lorsque l'on parle de déterminisme du sexe, la première chose qui vient à l'esprit de la plupart des gens (biologistes ou non) est le système chromosomique XY, qu'on retrouve chez l'Homme (et la majorité des mammifères) et chez l'emblématique mouche *Drosophila melanogaster*. Dans ce genre de système, le sexe d'un individu est déterminé lors de la fécondation par son complément en chromosomes sexuels : les mâles possèdent deux chromosomes sexuels différents X et Y (le sexe mâle est le sexe hétérogamétique, qui produit deux types de gamètes). Les femelles, quant à elles, arborent deux chromosomes X (elles constituent le sexe homogamétique). Cependant, l'hétérogamétie mâle est loin d'être le seul système existant, et en réalité la **diversité des systèmes de déterminisme du sexe** chez les animaux et les plantes est absolument remarquable (voir Bachtrog et al., 2014 et Beukeboom & Perrin, 2014 pour des revues récentes).

Classiquement, les systèmes de déterminisme du sexe ont été rangés dans deux grandes catégories (Bull 1983). Parmi les **déterminismes génétiques** (GSD pour « genetic sex determination »), on trouve en plus de l'hétérogamétie mâle, l'hétérogamétie femelle ZZ/ZW, présente notamment chez les oiseaux, les serpents et les papillons. Il existe également des systèmes « dérivés » de ces systèmes hétérogamétiques classique, tels que le déterminisme polygénique où plusieurs déterminants génétiques (mâles et/ou femelles) ségrégent au sein d'une même espèce ou population, comme par exemple chez des cichlidés

africains (Ser et al. 2011; Moore and Roberts 2013) ou le xénope *Xenopus tropicalis* (Roco et al. 2015). Un autre exemple est le système XX/X0 où les mâles ne possède qu'un seul chromosome sexuel X, qu'on retrouve notamment chez le nématode *Caenorhabditis elegans*. Certaines mousses et algues (par exemple au sein du genre *Ectocarpus* ; Ahmed et al. 2014) possèdent un système haploïde U/V, où l'expression du sexe a lieu à la phase haploïde par le biais des chromosomes U (femelle) et V (mâle). D'autres GSD, moins fréquents, n'impliquent pas une unique paire de chromosomes mais l'ensemble du génome : c'est le cas de l'haplodiploïdie, présente chez les hyménoptères sociaux, où les mâles sont issus d'œufs non fécondés et sont donc haploïdes (alors que les femelles sont diploïdes), et du déterminisme qualifié d'élimination du génome paternel (paternal genome elimination), trouvé entre autres chez des cochenilles, où les mâles ne portent que le génome maternel suite à la perte du génome paternel (Gardner and Ross 2014).

Chez d'autres espèces, le facteur déterminant n'est pas génétique mais **épigénétique** (ESD), c'est-à-dire qu'un même génotype peut potentiellement engendrer un individu de sexe mâle ou femelle. Parmi les déterminants épigénétiques, on retrouve les déterminismes environnementaux (qui impliquent des stimuli externes tels que la température chez des reptiles, la photopériode chez des crustacés ou l'environnement social chez des poissons), et la manipulation parasitaire (par exemple la féminisation provoquée par *Wolbachia*, endosymbionte de nombreux arthropodes (Cordaux et al. 2011).

Cette dichotomie entre déterminismes génétiques et environnementaux est en train de s'effriter un peu dans la mesure où de plus en plus de cas de **systèmes mixtes** sont décrits, notamment chez les poissons et les reptiles. Au sein de certains lignées, GSD et ESD peuvent être vus comme les deux extrémités d'un continuum (Sarre et al. 2004). Dans ce cas, il arrive que le sexe soit déterminé par une combinaison de facteurs génétiques et environnementaux, comme par exemple chez le lézard *Pogona vitticeps*, dont le sexe est déterminé par un système ZZ/ZW en dessous de 32°C, mais au-dessus, les individus ZZ (normalement mâles) ont tendance à se développer en femelles (Ezaz et al. 2005; Quinn et al. 2007), ou chez le Tilapia du Nil, où le sexe d'un individu résulte d'une complexe interaction entre des facteurs génétiques (dits majeurs et mineurs) et environnementaux (température) (Baroiller et al. 2009).

B. Evolution du déterminisme du sexe

Bien entendu, s'il existe une telle diversité de systèmes de déterminisme du sexe, cela implique que ces systèmes ne sont pas figés, et que des transitions d'un système à un autre peuvent se produire. Le mode de déterminisme sexuel est décrit chez de plus en plus d'espèces, notamment grâce au développement de techniques permettant de rapidement caractériser des systèmes à déterminisme chromosomique (méthodes basés sur le séquençage à haut débit, tels que le séquençage RAD-seq (Baird et al. 2008; Gamble and Zarkower 2014). L'intégration de toutes ces données dans un cadre phylogénétique (grâce entre autres aux efforts du « Tree of sex consortium », dont l'objectif est de compiler toutes les données disponibles concernant les systèmes de déterminisme du sexe connus (Bachtrog et al. 2014; Tree Of Sex Consortium et al. 2014)) permet de mieux comprendre de nombreux aspects de l'évolution du déterminisme du sexe et révèle à quel point c'est un trait dynamique dans de nombreuses lignées.

a. Les Rythmes de transitions

Au sein de nombreux taxons, les systèmes de déterminisme du sexe sont variés et **les transitions d'un système à un autre fréquentes**. A des échelles taxonomiques larges d'abord, chez les poissons téléostéens par exemple, on retrouve quasiment tous les modes de déterminisme du sexe cités précédemment (mis à part l'haplodiploidie et le paternal genome elimination, trouvé uniquement au sein de quelques lignées d'invertébrés, principalement des arthropodes). Parmi les espèces à sexes séparés, il existe des espèces à GSD (hétérogamétie mâle, hétérogamétie femelle, systèmes polygéniques), d'autres à ESD (température, pH) et des systèmes mixtes (Devlin and Nagahama 2002; Mank and Avise 2009). Autre exemple récent, chez les diptères (mouches et moustiques), une douzaines de systèmes chromosomiques différents ont été identifiés parmi 37 espèces, réparties dans 22 familles, impliquant l'existence de transitions régulières au sein de ce groupe (Vicoso and Bachtrog 2015). A une échelle taxonomique plus fine, au sein de la famille des Geckos, il existe des espèces à hétérogamétie mâle, d'autres à hétérogamétie femelle et d'autres encore dont le sexe est dépendant de la température, et il a été suggéré qu'entre 17 et 25 transitions d'un système à un autre ont dû avoir lieu au sein de ce clade (Gamble et al. 2015). On pourrait descendre l'échelle taxonomique petit à petit, les exemples fleurissent avec l'accumulation de données mentionnée plus haut, et révèlent une surprenante diversité dans de nombreux clades, diversité qui s'exprime même au niveau spécifique dans certains

cas. Il existe en effet des espèces au sein desquelles différentes populations arborent des modes de déterminisme du sexe différents. C'est le cas chez la mouche *Musca domestica* ou la grenouille rousse *Rana rugosa*, chez qui différentes paires de chromosomes font office de chromosomes sexuels d'une population à l'autre au sein de la même espèce. (Dubendorfer et al., 1992; Uno et al., 2008).

Contrairement aux taxons cités précédemment, **il en existe d'autres au sein desquels le déterminisme est très conservé**, c'est le cas des serpents, des papillons, des oiseaux et des mammifères thériens. Tous possèdent des systèmes hétérogamétiques (ZW pour les trois premiers et XY pour les derniers), qui ont évolué indépendamment il y a plus de 100 millions d'années (Matsubara et al. 2006; Nam and Ellegren 2008; Veyrunes et al. 2008; Sahara et al. 2012). Comment expliquer cette préservation, qui contraste grandement avec le dynamisme évoqué dans le paragraphe précèdent ? Tous ces systèmes anciens ont un point commun : un déterminisme sexuel hétérogamétique. Nous avons notre coupable. La conservation de ces systèmes est en effet inhérente à l'évolution des chromosomes sexuels.

b. Evolution des chromosomes sexuels

Structurellement, les chromosomes sexuels chez l'Homme sont **hétéromorphes**. Observés sur un caryotype, le chromosome X a une taille comparable à celle de la plupart des chromosomes non sexuels (les autosomes) alors que le chromosome Y est beaucoup plus petit. Si c'est aussi le cas chez les autres mammifères thériens, c'est loin d'être une généralité chez les espèces à déterminisme chromosomique. La différence de taille entre le Z et le W est par exemple variable chez les oiseaux et les serpents, et chez d'autres espèces, les chromosomes sexuels sont même homomorphes, c'est-à-dire indistinguables caryologiquement (la présence de chromosomes sexuels est alors mise en évidence par des expériences de réversion du sexe artificielle). L'observation d'espèces sans chromosomes sexuels, et d'autres avec des chromosomes sexuels homomorphes ou hétéromorphes (figure 1), a été interprété comme étant la preuve que ces états représentent différentes étapes d'un continuum (Ohno 1967), que les chromosomes sexuels dérivent d'une paire d'autosomes classique (suite à l'apparition d'une mutation contrôlant le déterminisme du sexe), et qu'il ont tendance à se différentier avec le temps.

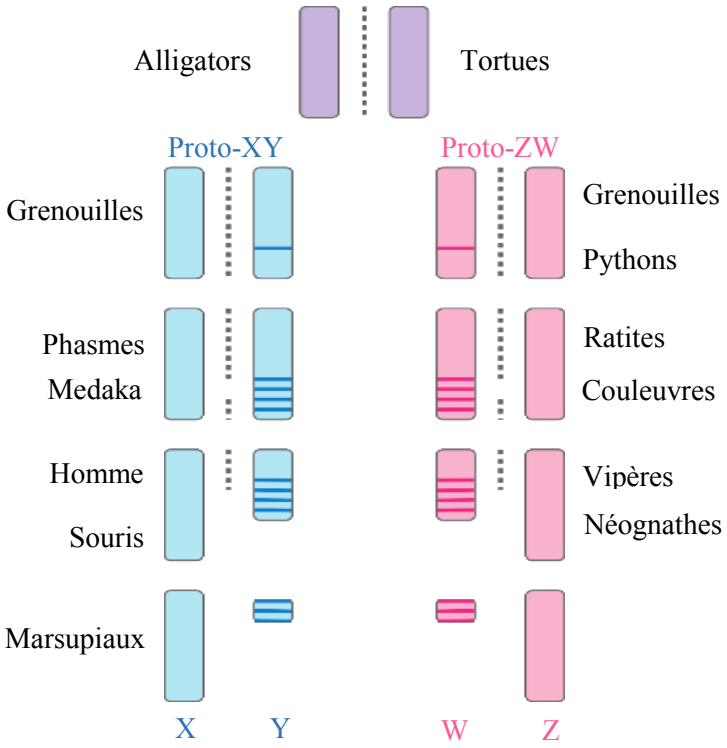

Figure 1. Variabilité dans la différentiation des chromosomes sexuels chez différents groupes de vertébrés.

Les **patrons génétiques de différentiation** des chromosomes sexuels et **les forces évolutives** impliquées dans cette différentiation ont fait l'objet de recherches approfondies et ont été décrits dans de nombreuses articles de revue (e.g. : Charlesworth 1991; Charlesworth and Charlesworth 2000; Bachtrog 2006, 2013; Marshall Graves 2006; Bergero and Charlesworth 2009…). Rapidement, dans les systèmes XY, le processus de différentiation est initié par l'émergence d'un allèle de déterminisme mâle sur un des deux membres d'une paire d'autosomes, qui devient le proto-Y. Si des allèles à effet sexuellement antagoniste mâles[1] se trouvent à proximité du gène de déterminisme, il y aura une forte pression de sélection pour que ces allèles et ce gène co-ségrègent. Un arrêt de la recombinaison entre le X et le Y (alors que le X continue à recombiner avec son homologue chez les femelles) se produit alors, menant à l'apparition d'une région sexe-spécifique sur le Y. Cette zone non-recombinante a tendance à s'agrandir avec le temps et l'accumulation

[1] les gènes ou allèles à effet sexuellement antagonistes ont un effet bénéfique dans un sexe et détrimental dans l'autre, Rice 1984)

de gènes et allèles à effet sexuellement antagoniste, réduisant progressivement la taille de la zone recombinante. Cette absence de recombinaison à des effets forts sur l'évolution des deux chromosomes sexuels, qui ont tendance à prendre des trajectoires évolutives drastiquement différentes. Plusieurs facteurs sont impliqués dans cette différentiation, et leurs effets sont résumés dans la table 1. (A noter que les mêmes conséquences sont également attendues pour les chromosomes Z et W).

	Y (zone non recombinante)			**X**		
Facteur	↓ taille efficace (1/4)	Transmission unilatérale	↑ liaison génétique	↓ taille efficace (3/4)	Transmission biaisée (2/3 ♀ - 1/3 ♂)	Exposition hémizygote chez les ♂
Conséquences	↑ dérive	Accumulation de : -gènes SA -distorteurs de transmission	↑ Effets Hill-Robertson	↑ dérive (moindre par rapport au Y)	Accumulation de gènes à effet SA	Accumulation d'allèles récessifs avantageux pour les ♂
Résume	Erosion génétique et accumulation de gènes SA favorisant le mâle			Evolution rapide et accumulation de gènes SA impliqués dans la différentiation sexuelle		

Table 1 : Facteurs impliqués dans l'évolution des chromosomes sexuels (ici hétérogamétie mâle). SA : sexuellement antagoniste. (sources: Beukeboom & Perrin, 2014; Vallender & Lahn, 2004).

Ainsi, le raccourcissement du chromosome Y est expliqué par une érosion génétique (accumulation de mutations délétères, perte de gènes…) due à la réduction de sa taille efficace et aux effets Hill-Robertson[2]. Certains chromosomes hétérogamétiques ont perdu la plupart de leur contenu en gènes (comme c'est le cas chez les mammifères) et les gènes qui échappent à cette érosion sont généralement indispensables à la fonction mâle, leur intégrité étant maintenue grâce à des phénomènes tels que la conversion génique intra-chromosomique (Mank 2012). Le chromosome X n'est pas en reste puisque son contenu en gènes est lui aussi affecté par l'arrêt de recombinaison et son statut hémizygote le rend notamment sujet à l'accumulation de mutations récessives bénéfiques pour les mâles. Cependant le phénomène le plus remarquable qui affecte le chromosome homogamétique

[2] L'influence des différents effets Hill-Robertson sur la dégénérescence du Y est résumée dans (Bachtrog 2006)

est la compensation de dosage (revues dans Mank 2009, 2013; Disteche 2012). La dégradation du Y créé un déséquilibre de dosage des gènes du X: les femelles possèdent deux copies des gènes portés par ce chromosome, et les mâle une seule, mise à part dans la zone pseudo-autosomale. Ce phénomène induit des différences de niveau de transcription des gènes concernés, mais également de nombreux gènes autosomaux dont l'expression dépend de gènes sur les chromosomes sexuels. La compensation de dosage permet d'égaliser l'expression des gènes portés par le X chez les mâles et les femelles et se fait par le doublement de la transcription des gènes concernés sur le X chez le sexe hétérogamétique, et/ou par l'intermédiaire de l'inactivation d'un des chromosomes X chez le sexe homogamétique.

Pour faire le lien avec la partie précédente traitant des rythmes de transitions, il est intéressant de noter que dans les lignées où le déterminisme du sexe est très conservé (mammifères, oiseaux…), les chromosomes sexuels sont (en général) extrêmement différentiés, et que cette forte différentiation et les adaptations associés (tel que la compensation de dosage, l'accumulation de gènes sexe-spécifiques) représentent un frein pour les transitions (production d'embryons non-viables, disruptions de la méiose.. ces systèmes sont considérés comme des pièges évolutifs, ou "evolutionary traps", Pokorná and Kratochvíl 2009).

D'autre part, dans les systèmes où les chromosomes sexuels sont homomorphes, les premiers pas de la dégénérescence du chromosome hétérogamétique pourrait au contraire favoriser les transitions, à condition que le coût de l'érosion génétique soit plus fort que les bénéfices apportés par l'arrêt de la recombinaison (Blaser et al. 2012).

c. Causes ultimes des transitions

Eviter le coût de la dégénérescence du chromosome hétérogamétique n'est bien sûr pas le seul processus qui peut provoquer une transition d'un système du déterminisme du sexe à un autre. Les mécanismes évolutifs pouvant être impliqués dans ces transitions sont variés et ont fait l'objet d'un grand nombre d'études théoriques. Ils seront présentés ici dans les grandes lignes, basé sur les revues de la littératures faites par Beukeboom et Perrin (2014) et van Doorn (2013, 2014), et en mettant l'accent sur les transitions impliquant des systèmes de déterminisme chromosomiques.

***Processus neutres**

Bull et Charnov sont les premiers à analyser mathématiquement les conditions permettant le passage d'un système de déterminisme chromosomique à un autre (1977), et mettent en évidence qu'une telle transition peut se produire à cause du simple effet de la dérive génétique. Grace à un système d'équations de récurrence, ils montrent que lorsque deux gènes de déterminisme du sexe co-ségrègent dans une population finie, et que ces gènes n'ont pas d'effet sur la valeur sélective, il existe une infinité d'équilibres neutres stables où les deux facteurs de déterminisme sexuel coexistent. Les fréquences des différents génotypes sont alors seulement soumises à la dérive génétique, qui pourrait conduire à la fixation de l'un ou l'autre des facteurs. Ainsi, si par exemple une mutation féminisante dominante (F) apparait dans un système XX/XY sur le chromosome X, et que les nouveaux génotypes formés : femelles FY et XF et mâles YY ont la même valeur sélective que les individus XX et XY respectivement, le système sera dans un état polygénique jusqu'à ce qu'un des deux facteurs de déterminisme Y ou F se fixe sous l'effet de la dérive. L'hypothèse selon laquelle tous les génotypes ont une fitness égale est cependant assez peu réaliste, de plus une mutation émergente, initialement rare, aura plus de chance d'être perdue par dérive. La dérive génétique joue donc probablement un rôle mineur dans les transitions d'un système de déterminisme sexuel à un autre.

***Avantage sélectif au(x) génotype(s) émergent(s)**

Bull et Charnov ont aussi analysé les conditions permettant une telle transition lorsqu'un des génotypes émergents (FY, XY et YY dans l'exemple précédant) possède une fitness supérieure ou inférieure aux génotypes préexistants (XX et XY). Les résultats de leurs simulations sont assez intuitifs et montrent que l'augmentation de la fitness des porteurs de la mutation émergente facilitera son invasion et sa fixation et qu'au contraire, une fitness moins bonne des « néo-genotypes » provoquera son élimination. En d'autres termes, une transition peut se produire si la mutation féminisante ou masculinisante a un effet positif sur la valeur sélective des individus qui la portent, soit grâce à des effets pléiotropes ou à un déséquilibre de liaison avec des gènes sous sélection, comme par exemple des gènes à effet sexuellement antagonistes (voir modèles par van Doorn and Kirkpatrick ; 2007, 2010).

***Sélection sur le sex-ratio optimal**

Le principe de Fisher (1930) montre que le sex-ratio évolutivement stable dans une population est généralement de 1:1, et que, dans ces conditions, les individus produisant un progéniture avec un sex-ratio équilibré sont avantagés. Il semblerait que la prépondérance des systèmes de déterminisme hétérogamétiques puisse être expliqué par ce principe, car la ségrégation aléatoire des chromosomes sexuels lors de la méiose est un moyen fiable d'assurer la production d'autant de descendants des deux sexes (Uller et al. 2007). Cependant, la sélection pour un sex-ratio équilibré n'est pas universelle. Certains facteurs peuvent favoriser la production d'un excès de mâles ou de femelles, comme c'est le cas de la sélection interdémique dans une population structurée qui sélectionne pour la production d'un excès de femelles (Wilson and Colwell 1981). Ainsi, Vuilleumier et ses collaborateurs (2007) ont mis en évidence théoriquement que la sélection interdémique pouvait favoriser l'invasion d'un chromosome W dans un système à hétérogamétie mâle, pouvant mener à une transition vers un système à hétérogamétie femelle. Par ailleurs, il arrive aussi que le sex-ratio optimal fluctue avec certaines conditions environnementales, telles que le taux d'accouplement entre apparentés ou la compétition locale pour l'accès aux partenaires ou aux ressources (Hamilton 1967; Charnov 1982; Bull and Charnov 1988). Dans ces conditions, un système de déterminisme du sexe permettant à la mère de manipuler de manière adaptative le sex-ratio de sa progéniture sera avantagé, et la sélection favorisera des systèmes tels que l'haplodiploidie, où la proportion de mâles correspond à la proportion d'œufs non-fécondés, qui peut être sous contrôle maternel. Typiquement, les sex-ratios observés chez les hyménoptères à déterminisme haplodiploïde en compétition locale pour l'accès aux partenaires collent parfaitement avec les prédictions théoriques (Shuker and West 2004).

***Conflit génomiques**

Différents éléments génétiques (dans deux génomes distints, ou différentes portions du même génome) peuvent avoir des intérets divergents en termes de transmission et donc rentrer en conflit (Burt and Trivers 2006). Il a été suggéré que les conflits génomiques sont sans doute le facteur majeur pour expliquer la diversité des systèmes de déterminisme du sexe (Werren and Beukeboom 1998). Les conflits sont souvent générés par des élements génétiques dit « égoïstes », qui ont la caractéristique d'augmenter leur propre transmission, au détriment des autres éléments génétiques en jeu. Par exemple, dans un système

hétérogamétique, un élément génétique égoïste ayant un impact sur le ratio de transmission des chromosomes sexuels (c'est le cas des distorteurs de transmission sur les chromosomes sexuels où de certains parasites intracellulaires à transmission uniparentale), va rentrer en conflit avec le reste du génome et notamment avec le chromosome sexuel le moins transmis (Hamilton 1967). Par conséquent, la sélection favorisera tout élement permettant de revenir au sex-ratio à l'équilibre, et il a été montré qu'une des manières d'y parvenir est la modification du système du déterminisme du sexe (Kozielska et al. 2010). Ainsi, suivant l'invasion d'un élément génétique égoïste biaisant le sex-ratio en faveur des mâles, un gène de déterminisme du sexe femelle (convertissant des mâles en femelles) sera automatiquement sélectionné et pourra mener à une modification du système de déterminisme du sexe, accompagnée en général de la perte de l'élément égoïste.

d. Modèles biologiques adaptés à l'étude de ces causes ultimes

Malgré que les causes ultimes des transitions aient fait l'objet de nombreuses études théoriques, en pratique elles sont compliquées à déterminer, en témoigne le nombre réduit d'exemples où les mécanismes impliqués sont clairement identifiés. Cette difficulté est liée au fait que les transitions sont en général rapides, et qu'une fois que la transition est achevée, il est devient compliqué d'identifier la cause de l'invasion de la mutation impliquée, surtout lorsqu'il s'agit d'une transition entre deux systèmes hétérogamétiques. En effet, l'hypothèse d'un avantage sélectif associé à la mutation n'est plus testable dans la mesure où les anciens génotypes ont disparu, ou comme dit précédemment, le conflit génomique responsable est en général résolu lors de transition, en conséquent l'effet d'un potentiel élément génétique égoïste n'est plus détectable une fois la transition achevée.

Deux cas de figures sont favorables à l'identification de ces causes ultimes:

(i) **observer la transition en direct** : Chez le Lézard *Pogona vitticeps*, une transition d'un système de déterminisme du sexe chromosomique (hétérogamétie femelle) à un système où le seul facteur déterminant est la température est en cours (des données temporelles montrent la décroissance de la fréquence du chromosome W en milieu naturel, Holleley et al. 2015). Il a été démontré expérimentalement que deux facteurs sont impliqués dans cette transition. D'une part la sensibilité à la température du système ancestral (au-dessus d'une certaine température, les individus ZZ deviennent des femelles), et d'autre part que ces femelles ZZ pondent plus d'œufs que les femelles ZW, ce qui résulte sans doute en une meilleure fitness en milieu naturel. Si la transition s'achevait en milieu naturel,

avec la disparition du chromosome W, il aurait été impossible d'identifié ces deux causes *a posteriori*.

(ii) **étudier des systèmes polygéniques**, où plusieurs facteurs impliqués dans la détermination du sexe co-ségrègent au sein d'une population. Ces systèmes sont rares et on pourrait argumenter sur le fait qu'un système polygénique n'est rien d'autre qu'un état transitoire entre deux systèmes de déterminisme hétérogamétiques (Rice and May 2007), et que leur étude revient au cas (i). Cependant, il existe des systèmes polygéniques évolutivement stables, dans le cas de figure où les individus portant un génotype intermédiaire[3] ont un avantage sélectif (Bull and Charnov 1977), et d'autres pour lesquels le polymorphisme est maintenu tout simplement car la transition ne peut pas être achevée à cause de contraintes liées à la différentiation des chromosomes sexuels. C'est le cas chez quelques espèces de mammifères où un élément féminisant est apparu sur le chromosome X, ces systèmes sont détaillés dans la prochaine partie.

Pour résumer, les systèmes biologiques pertinants pour étudier les facteurs évolutifs responsables des transitions d'un déterminisme du sexe à un autre sont rares et, contre toute attente, certains mammifères pourraient faire de bon modèles pour mieux comprendre les mécanismes impliqués.

2. Le déterminisme du sexe chez les mammifères

A. Histoire évolutive des chromosomes sexuels et mécanismes moléculaires

Comme dit précédemment, le déterminisme du sexe chez les mammifères thériens (hétérogamétie mâle) est ancien et montre très peu de variations.

- **Quand les chromosomes sexuels sont-ils apparus ?**

Les chromosomes X des marsupiaux et des placentaires sont en grande partie homologues, cependant, ils ne partagent aucune homologie avec les chromosomes sexuels de leurs cousins monotrèmes (Veyrunes et al. 2008), mettant en évidence que les chromosomes sexuels des thériens sont apparus après la divergence avec les monotrèmes

[3] Par exemple les femelles WX dans une transitions XY/XX -> ZZ/ZW

(estimée à 166 millions d'années) et avant celle des marsupiaux et placentaires (148 millions d'années) (Van Rheede et al. 2006; Bininda-Emonds et al. 2007).

- **Comment la différentiation en mâle ou femelle est-elle régulée ?**

Après la mise en évidence dans les années 50 que le chromosome Y devait porter un gène responsable du développement des gonades mâles, le gène *Sry* (pour Sex-determining region of the Y) a été découvert suite à plusieurs décennies de recherche dans la partie non recombinante de ce chromosome (Sinclair et al. 1990). Son expression (pic à 11,5 jours post-fécondation chez la souris) initie la cascade génique de différentiation mâle en activant *Sox9,* qui lui-même régule l'expression d'autres gènes, qui induisent le développement des gonades indifférenciées de l'embryon en testicules. En l'absence de *Sry*, des gènes tels que *Wnt4, Rspo1* et *Foxl2* sont exprimés et induisent le développement d'ovaires (figure 2).

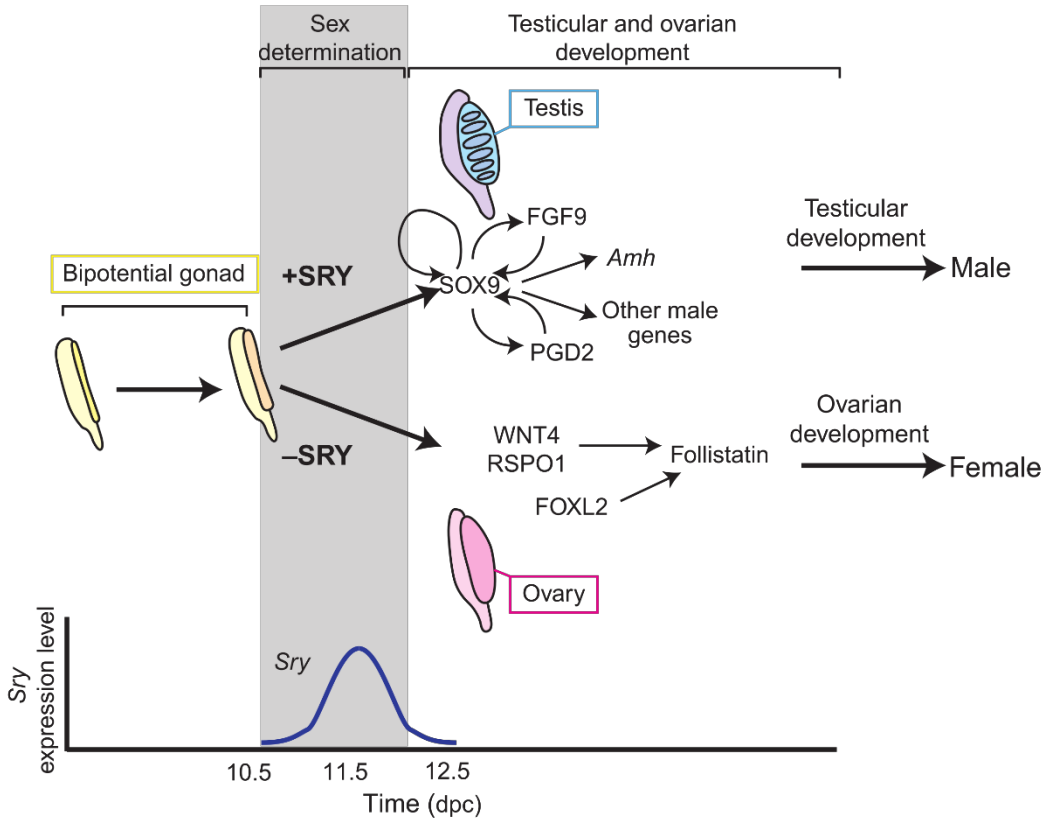

Figure 2. Représentation schématique de la différentiation sexuelle chez les mammifères. Le nombre de gènes impliqués dans la cascade étant très important, ils ne sont pas tous représentés ici (voir revue de la littérature par Wainwright & Wilhelm (2010) pour plus de détails).

Figure adaptée de Kashimada & Koopman (2010).

- **A quel point le X et le Y des mammifères thériens sont-ils différenciés ?**

Sans doute au moment où le gène *Sry* est apparu, le X et le Y se sont mis à diverger. Ces quelques 150 millions d'années de divergence ont rendu les deux chromosomes sexuels extrêmement différents (Marshall Graves 2006). Chez la grande majorité des mammifères, le Y est petit, contient de nombreuses séquences répétées et est très pauvre en gènes. Par exemple, le chromosome Y de la souris n'a conservé que 9 gènes sur les 639 que contenait la paire d'autosomes ancestrale. Seuls des gènes impliqués dans la fonction mâle ont été épargnés par la dégénérescence de la région non recombinante, et quelques autres gènes ont été acquis par des évènements de translocation (Bellott et al. 2014; Soh et al. 2014). Cette dégénérescence n'a d'ailleurs pas été graduelle mais ponctuée par la perte successive de la recombinaison dans des régions qu'on appelle des strates évolutives (Lahn and Page 1999). Sur le chromosome X par contre, la plupart des gènes ancestraux ont été conservés (97% sur le X de l'Homme ; Mueller et al. 2013). Néanmoins, ce chromosome a aussi un contenu en gènes biaisé : il est enrichi en gènes impliqués dans la reproduction (Saifi and Chandra 1999; Wang et al. 2001; Khil et al. 2004), et dans le développement et fonctionnement du cerveau (Zechner et al. 2001; Xu and Andreassi 2011).

- **Qu'est ce qui fait que le système est si conservé ?**

Comme évoqué précédemment, les systèmes de déterminisme hétérogamétiques sont considérés comme des pièges évolutifs (Pokorná and Kratochvíl 2009). Chez les mammifères thériens, la compensation de dosage ainsi que l'évolution d'un contenu en gène hautement spécialisé représentent une véritable barrière aux transitions. La mutation d'un gène impliqué dans la cascade de déterminisme sexuel abouti généralement à une inversion du sexe (femelle XY ou mâle XX) où à un individu qui possède un mélange de caractères sexuels mâle et femelle (Jiménez et al. 2013). Alors que ce genre d'inversion du sexe n'a peu (voir pas du tout) de conséquences sur le succès reproducteur dans les espèces à chromosomes sexuels peu différentiés (e.g. Nanda et al. 2003), les conséquences chez la plupart des mammifères sont dramatiques, et mènent généralement à la stérilité. Par exemple chez une femelle XY, la baisse de fitness est liée à la production d'embryons YY non-viables, et, à cause de la spécialisation du contenu en gènes, elle subira également les effets délétères de l'absence de deux chromosomes X et de la présence du chromosome Y (entrainant une perte d'oocytes lors de la méiose et un mortalité embryonnaire accrue, revue dans Vernet et al. 2014) .

- **Donc absolument tous les mammifères thériens ont le même système XX/XY ?**

Globalement, la vision de systèmes hétérogamétiques ayant un destin tragique et irrémédiable a quelque peu changé ces dernières années et la communauté scientifique s'est rendue compte que ces systèmes ne sont pas si figés. Ainsi, tous les chromosomes sexuels ne dégénèrent pas, comme en témoignent les chromosomes sexuels indifférenciés des pythons et des ratites (pourtant apparus il y a 140 et 120 millions d'années respectivement ; Vicoso et al., 2013a; Vicoso et al., 2013b). D'autre part, le système XY des mammifères n'est pas si contraint qu'il en a l'air : certaines espèces possèdent des néo-chromosomes sexuels (qui résultent d'une fusion entre une paire d'autosomes et les chromosomes sexuels, e.g. Zhou et al. 2008) et plusieurs espèces de rongeurs présentent des systèmes chromosomiques atypiques.

B. Les déterminismes atypiques

Certains mammifères ont réussi à contourner les contraintes imposées par les chromosomes sexuels différenciés et ont aujourd'hui des **systèmes de déterminisme du sexe dit atypiques** (Fredga 1994, table 2).

Espèce	♀	♂	réferences
Ellobius tancrei	XX	XX	(Just et al. 1995)
Ellobius lutescens	X0	X0	(Just et al. 1995)
Tokudaia osimensis	X0	X0	(Honda and Itoh 1977)
Mus triton	X0	X0	(Jotterand-Bellomo 1988)
Microtus oregoni	X0	XY	(Ohno et al. 1963)
Acomys selousi	X0	XY	(Matthey 1965)
Akodon sp.	XX, XX*, X*Y	XY	(Bianchi 2002)
Dicrostonyx torquatus	XX, XX*, X*Y	XY	(Fredga et al. 1976)
Myopus schisticolor	XX, XX*, X*Y	XY	(Fredga et al. 1976)
Mus minutoides	XX, XX*, X*Y	XY	(Veyrunes et al. 2010)

Table 2. Liste des espèces de mammifères ayant un déterminisme du sexe atypique.

Globalement, ces systèmes sont pour l'heure mal compris, et les mécanismes moléculaires impliqués restent largement inconnus, mais les espèces concernées peuvent être classées en quatre catégories (XX/XX - X0/X0 - X0/XY - XX,XY/XY).

Au sein des deux premières catégories, le chromosome Y a complétement disparu. Chez les campagnols *Ellobius tancrei* et *Ellobius lutescens*, ainsi que chez le rat épineux *Tokudaia osimensis*, il a été montré que le gène de déterminisme mâle *Sry* n'assure plus son rôle de d'initiateur dans le déterminisme du sexe, puisqu'il a disparu avec le chromosome Y (Just et al. 1995; Soullier et al. 1998). Le(s) gène(s) ayant pris le relai n'a (ont) toujours pas été identifié(s). Dans les deux autres catégories, le chromosome Y est toujours présent, et seules les femelles possèdent un complément en chromosomes sexuels non conventionnel. Chez le campagnol *Microtus oregoni*, la présence d'un seul chromosome sexuel X chez les femelles est lié au fait que le chromosome X est absent des cellules de la lignée germinale mâle (Ohno et al. 1963). Au sein de plusieurs espèces du genre *Akodon*, chez les lemmings *Dicrostonyx torquatus* et *Myopus schisticolor* et chez la souris naine Africaine *Mus minutoides*, il existe des femelles avec un complément en chromosome sexuels XY (Fredga et al. 1976; Bianchi 2002; Veyrunes et al. 2010). Il a été montré chez ces quatre espèces que cette inversion du sexe n'est pas liée à une mutation sur le chromosome Y, mais à l'apparition d'un gène déterminant du sexe femelle dominant sur le chromosome X (le chromosome est alors nommé X*), ce qui en fait des espèces à déterminisme sexuel **polygénique**. Ainsi, alors que tous les mâles ont le même complément en chromosomes sexuels, il existe trois types de femelles : les femelles standard XX, les femelles hétérozygotes XX* et les femelles hémizygotes X*Y. Ce système est apparu au moins quatre fois de manière indépendante, et il est possible que la même mutation soit impliquée dans la modification du déterminisme sexuel.

Est-ce que toutes ces espèces ont quelque chose en commun ? Pour commencer, toutes appartiennent à la famille des rongeurs *Muridae* (qui représente tout de même plus de 20% des espèces actuelles de mammifères thériens). Deux points ont été avancés pour expliquer pourquoi des modification du système de déterminisme du sexe ne sont observées que chez ces rongeurs : d'une part, ils semblent relativement tolérants aux accidents chromosomique impliquant les chromosomes sexuels (Fredga and Bulmer 1988), comme le souligne les nombreux réarrangements trouvé dans le sous-genre *Nannomys* (auquel appartiennent *Mus triton* et *Mus minutoides*, Veyrunes et al. 2004). D'autre part, plusieurs aspects de l'écologie et de la reproduction communs à de nombreux rongeurs (taux

reproductif élevé, fluctuations de densité de populations, comportement social favorisant la consanguinité…) pourraient favoriser la fixation de ces remaniements chromosomiques.

a. Les causes évolutives

La plupart de ces espèces n'ont reçu que très peu d'attention (*A. selousi, E. tancrei, E. lutescens, T. Osimensis, M. triton*), mais quelques études donnent des pistes pour comprendre les mécanismes évolutifs entrant en jeu dans l'évolution et le maintien de ces systèmes. D'une part, tous les individus ayant des compléments en chromosomes sexuels atypiques sont fertiles, et des mécanismes pour compenser le coût reproductif de la production d'embryons non-viables (YY, 00… voir table 3) ont évolué.

(a)

	X	0
X	†	X0
0	X0	†

(b)

	X	0
X	†	♀X0
Y	♂XY	†

(c)

	X
X	♀XX
Y	♂ XY

X	X*
♀XX	♀XX*
♂ XY	♀X*Y

X*	Y
♀XX*	♂ XY
♀X*Y	†

Table 3. Tableaux de croisement pour les espèces (a) X0/X0, (b) X0/XY et (c) XX-XX*-X*Y/XY. Les chromosomes transmis par les femelles sont en colonnes et les mâles en lignes.

Chez *Microtus oregoni*, par exemple, un phénomène de non-disjonction des chromosomes sexuels lors de la mitose dans la lignée germinale conduit à la production par les femelles de 100% d'oocytes portant un X et par les mâles de spermatozoïdes Y ou 0 en proportion 1:1, évitant ainsi le production d'embryon XX et Y0 (Ohno et al. 1963). De manière similaire, chez *Myopus schisticolor*, le Y des femelles X*Y est éliminé de la lignée germinale par un mécanisme de double non-disjonction méiotique dans l'ovaire fœtal, empêchant la production d'embryons YY (Winking et al. 1981). Il n'existe pas de mécanismes similaires chez *Akodon azarae* ou *Dicrostonyx torquatus*, cependant les femelles X*Y de ces espèces compensent la perte de fertilité attendue par d'autres moyens. Chez les deux espèces, les femelles sexe-reversées ont un taux d'ovulation plus fort que les femelles XX et XX* (Gileva et al. 1982; Espinosa and Vitullo 1996), et chez la seconde, il a même été montré que ces femelles ont une vie reproductive plus longue. Ces observations font bien sûr penser à l'hypothèse proposée par Bull et Charnov pour expliquer les transitions d'un déterminisme du sexe hétérogamétique à un autre (voir partie 1.B.c.), qui suggère qu'un avantage de succès reproducteur d'un des génotypes émergent (dans ce cas les femelles X*Y) pourrait entraîner la modification du déterminisme du sexe. La raison pour laquelle le X* ne se fixe pas est liée au fait que chez les mammifères le X « classique » ne peut pas disparaitre, étant donné qu'il reste indispensable pour faire des mâles.

D'autres mécanismes évolutifs ont été proposés pour expliquer la modification du déterminisme du sexe chez certains de ces mammifères. En relation avec l'hypothèse de sélection sur le sex-ratio optimal, des études théoriques ont montré que la mutation féminisante (X*) chez les lemmings aurait pu évoluer grâce à son effet sur le sex-ratio (production d'un biais en faveur des femelles). En effet, si la consanguinité est suffisamment forte (cela nécessite tout de même que 75% des femelles s'accouplent avec leur père ou leur frère) ou si la population est structurée en dèmes, alors les femelles portant le X* auront un avantage sélectif car elles produisent plus de femelles, et le X* sera favorisé (Maynard Smith and Stenseth 1978; Benenson 1983).

b. Les conséquences

Par ailleurs, la question des conséquences de la modification du déterminisme du sexe chez ces mammifères n'a jamais été abordée.

Il a été proposé que les systèmes de déterminisme polygénique pourrait voir émerger différentes classes au sein d'un même sexe, où des individus avec le même sexe gonadique mais possédant des compléments en chromosomes sexuels différents pourraient avoir des caractères sexuels secondaires drastiquement différents (cela n'a jusqu'à maintenant jamais été vérifié, Moore and Roberts 2013). C'est d'autant plus vrai chez les mammifères avec ce type de déterminisme lorsqu'on prend en compte le fait que le chromosome X possède un excès de gènes impliqués dans la reproduction, dans le développement du cerveau et dans des fonctions cognitives (Hurst and Randerson 1999; Skuse 2005, 2006). Des différences phénotypiques, notamment comportementales (stratégies de reproduction, interactions sociales), entre les femelles XX, XX* et X*Y chez les quatre espèces de mammifères possédant des déterminismes polygéniques pourraient avoir des conséquences évolutives et écologiques importantes.

A un autre niveau, on pourrait également s'attendre à ce que l'architecture génomique si particulière des chromosomes sexuels soit affectée par ces modifications de déterminisme du sexe. En effet, l'apparition de nouveaux facteurs de déterminisme sexuels entraine des modifications des patrons de transmission des chromosomes sexuels, affectant l'effet des pressions de sélection qui ont façonné leur contenu. Comment évolue le contenu en gènes du chromosome X des espèces ayant perdu le chromosome Y, et quels sont les effets de l'arrêt de recombinaison du X dans les espèces X0/X0 ? Dans les espèces à déterminisme polygénique, le Y a perdu sa transmission mâle spécifique et la région portant la mutation féminisante sur le X* a sans doute acquis une transmission femelle spécifique, comment leur structure et contenu en gène évoluent-t-ils ? Ces questions restent pour le moment sans réponse.

Problématique

Les systèmes de déterminisme du sexe atypiques chez les mammifères apparaissent comme des modèles pertinentes pour mieux comprendre les causes et conséquences évolutives des transitions d'un système de déterminisme sexuel à un autre, cependant, ils restent largement inexplorés et mal compris. ***Mus minutoides***, la souris naine Africaine, qui est la dernière espèce à avoir intégré le club fermé des espèces à déterminisme atypique (Veyrunes et al., 2010), et qui appartient au même genre que la souris domestique (organisme modèle par excellence dans de nombreux domaines de recherche en biologie : génétique, comportement…), se présente comme le modèle idéal pour étudier les causes et conséquences évolutives d'une modification du déterminisme du sexe chez les mammifères.

1. *Mus minutoides*, la souris naine Africaine

La souris naine africaine appartiennent au sous-genre *Nannomys*, qui est le taxon le plus riche du genre Mus, avec 18 espèces (Musser and Carleton 2005). Leur nom leur vient de leur petite taille (de 3,5 à 12g), elles sont réparties dans toute l'Afrique sub-saharienne et occupent une large gamme d'habitats (Catzeflis and Denys 1992; Britton-Davidian et al. 2012). Les espèces du sous-genre *Nannomys* sont remarquables de par leur homogénéité morphologique qui contraste avec leur extraordinaire diversité chromosomique : elles possèdent de 16 à 36 chromosomes (18 à 36 chromosomes pour *Mus minutoides*, Veyrunes et al. 2014). Cette variabilité est liée à de fréquents remaniements chromosomiques, tel que des fusions robertsoniennes (fusions de deux chromosomes non-homologues par les centromères), des fusions sexe-autosomes, des fusions en tandem et des translocations de bras chromosomiques entiers (Revue dans Britton-Davidian et al. 2012). Le génome des *Nannomys* semblent être assez tolérant à l'apparition et la fixation de ce genre de remaniements complexes, pourtant habituellement rares et délétères. Ce n'est donc peut-être pas un hasard si on retrouve au sein de ce sous genre deux espèces ayant des déterminismes sexuels atypiques (*Mus minutoides* et *Mus triton*).

Mus minutoides est l'espèce la plus abondante et la plus largement repandue du sous-genre (Britton-Davidian et al. 2012), mais on ne sait presque rien de son écologie ou de ses traits d'histoire de vie (Skinner and Chimimba 2005). Elle vit dans des habitats variés (Monadjem 1999) et les densités de populations semblent très fluctuantes (Fichet-Calvet et al. 2009). Le déterminisme du sexe de cette espèce a été décrit en 2010, suite à la découverte

de femelles XY dans plusieurs populations en Afrique du Sud (Veyrunes et al. 2010). Cette inversion du sexe est génétique, mais le chromosome Y n'est pas impliqué : le gène du déterminisme mâle, *Sry* est en effet présent et fonctionnel chez les femelles XY. Par contre, des analyses caryotypiques ont révélé l'existence de deux types de chromosomes X, qui différent en terme de longueur et de structure. C'est donc en fait une mutation (toujours inconnue) sur le chromosome X qui empêche la masculinisation des embryons X*Y, et il existe dans cette espèce trois chromosomes sexuels : X, X* et Y, et leur combinaison forme quatre génotypes : les mâles XY et les femelles XX, XX* et X*Y (figure 3).

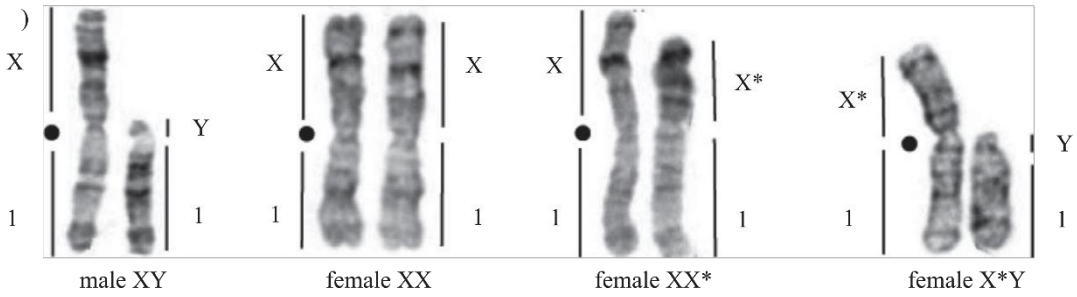

Figure 3. Chromosomes sexuels (G-banding) des mâles et des trois types de femelles chez *Mus minutoides*. Les chromosomes sexuels sont fusionnés à la paire d'autosome 1. Les points noirs montrent l'emplacement des centromères. Adapté de Veyrunes et al. 2010.

Le chromosome X* a été trouvé dans plusieurs localités de la large aire de distribution de l'espèce et des données de phylogéographie et de datation moléculaire (ADN mitochondrial et nucléaire) suggèrent que la mutation féminisante est apparue il y a au moins 0,9 millions d'années (Veyrunes et al. 2013). L'étendue de la zone recombinante sur le X* est inconnue mais plusieurs arguments suggèrent que la différentiation entre le X et le X* est en marche :

(i) Des études portant sur des système chromosomiques « jeunes » montrent que la différentiation des chromosomes sexuels peut être initiée rapidement, comme par exemple chez *Drosophila miranda* et son néo-Y (1-2 millions d'années[4]) qui montre des signes de dégénérescence (Bachtrog et al. 2008; Zhou and Bachtrog 2012).

(ii) Comme dit précédemment, des différences structurelles ont été observées entre le X et le X* : le G-banding montre que les deux chromosomes différent en terme de taille et en terme d'arrangement des bandes (voir figure 3). Des données préliminaires d'hybridations *in-situ* fluorescentes suggèrent l'existence de large remaniements de type inversion ou translocation (figure 4), et une autre analyse de cytogénétique, le aCGH (pour array CGH) a permis de détecter plusieurs régions qui varient en terme de nombre de copies, à causes d'amplifications ou de délétions (figure 5).

[4] Attention, les temps de génération des drosophiles et des souris ne sont bien sûr pas comparables.

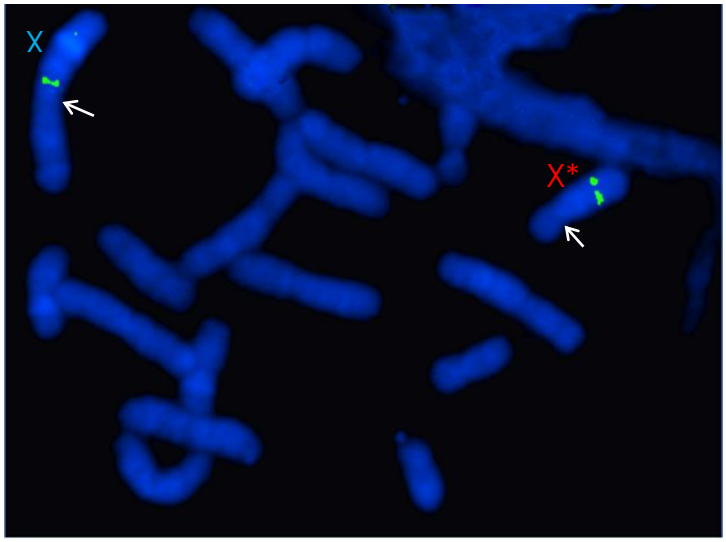

Figure 4 : Hybridation *in-situ* fluorescente d'une sonde BAC (Bacterial Artifical Chromosome, CH29-520L22, ~6.6 - 6.8 Mb) sur une métaphase d'une femelle XX*. Les flèches désignent l'emplacement des centromères des chromosomes sexuels. Le BAC s'hybride à proximité du centromère sur le X et de manière plus distale sur le X*.

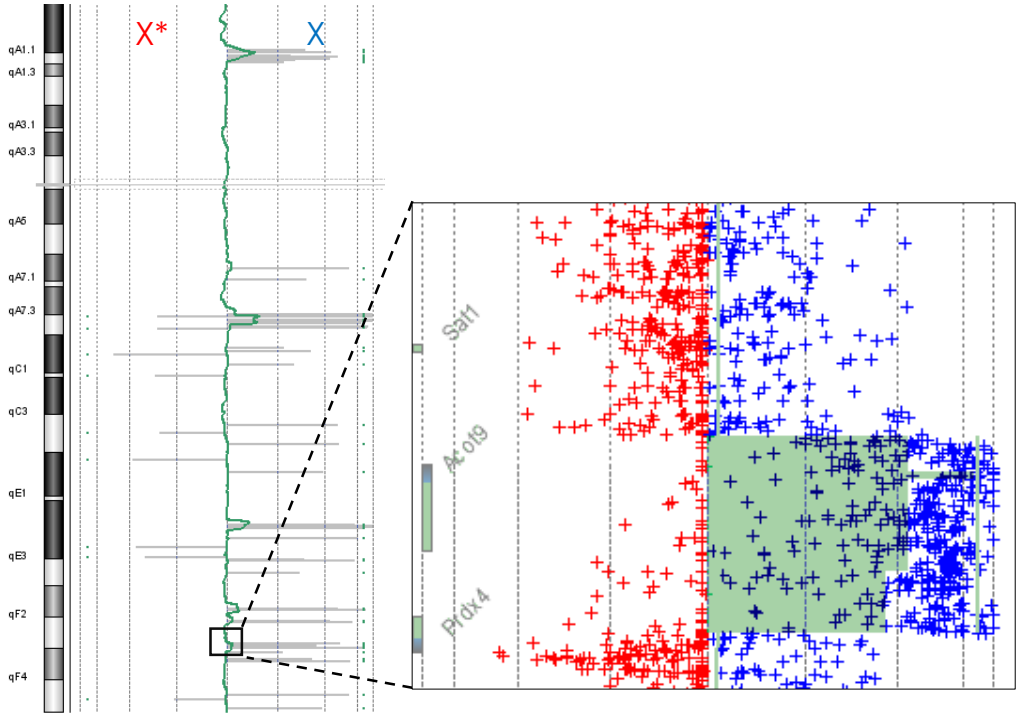

Figure 5 : résultats du aCGH, montrant l'existence de plusieurs régions mieux couvertes sur le X (suggérant l'existence de délétions sur le X*, ou d'amplifications sur le X), et zoom sur l'une de ces région.

2. Objectifs et plan de thèse

L'objectif de cette thèse est d'une part d'identifier les causes ultimes responsables de l'évolution du déterminisme du sexe atypique qu'on trouve chez la souris naine africaine, et d'autre part de comprendre les conséquences évolutives de l'apparition du chromosome X*.

Pour tenter de répondre à ces questions, j'ai mené une approche pluridisciplinaire (combinant des analyses comportementales, génomiques, théoriques, cytogénomiques, des analyses de traits d'histoire de vie…) basée sur des données récoltées au sein du 1er élevage au monde de *Mus minutoides* (mis en place en 2010 sur le campus de l'UM2).

Chapitre 1. Les causes de l'évolution de ce système

Deux hypothèses sont considérés pour expliquer l'apparition et le maintien du chromosome X* :

- Comme suggéré par Bull et Charnov (1977), une mutation féminisante sur le chromosome X peut augmenter en fréquence si les porteurs de la mutation ont un avantage sélectif. Ainsi, le X* pourrait avoir envahi si les femelles X*Y échappent à la perte de fertilité inhérente aux femelles XY chez les mammifères.
- Il a également été montré théoriquement qu'une modification du déterminisme du sexe peut se produire en réponse à l'invasion d'un élément génétique égoïste ayant un impact sur le sex-ratio (Kozielska et al. 2010). Dans ce cas de figure, le chromosome féminisant aurait pu apparaitre en réponse à l'invasion d'un élément génétique égoïste tel qu'un distorteur du chromosome Y, provoquant la production d'un excès de mâles.

Afin de tester ces deux hypothèses, une approche empirique a été combinée à une approche théorique : des données concernant **les traits d'histoire de vie** (manuscrit 1) et **le sex-ratio** (manuscrit 2) au sein des portées des trois types de femelles ont été récoltées, et des modèles mathématiques ont été développés pour tester différents **scénario évolutifs pour expliquer la transition** (manuscrit 2), en accord avec les résultats empiriques. Dans ce premier chapitre se trouve également une troisième partie, exposant des travaux qui découlent directement des découvertes concernant les analyses de sex-ratio, et j'y aborde **les causes proximales des biais de transmission des chromosomes sexuels observés chez les mâles**.

Chapitre 2. Les conséquences

Les conséquences d'une modification du système de déterminisme du sexe chez un mammifère n'ont jamais fait l'objet d'études quantitatives. Les conséquences de la mise en place du système de déterminisme polygénique chez *Mus minutoides* ont été évaluées sur le plan phénotypique et sur le plan de la génomique des chromosomes sexuels.

Ainsi, différent **traits phénotypiques**, comme **la morphologie** (manuscrit 4) et **le comportement** (Manuscrit 5), ont été investigués. D'autre part, avec l'apparition du chromosome X*, on s'attend à ce que les pressions de sélection façonnant l'évolution des chromosomes sexuels soient bouleversées et que les trajectoires évolutives des trois chromosomes X, X* et Y soient affectés. D'après la théorie, on pourrait notamment s'attendre à ce que le X et le X* cessent de recombiner et que le X* se mette à dégénérer. Le génome complet de la souris naine a été séquencé, et **la structure et la séquence des deux chromosomes ont été comparés** sur la base d'un alignement des données de séquençage sur le génome de référence de *Mus musculus* (Manuscrit 6).

CHAPITRE 1 : les causes ultimes de l'évolution du système de déterminisme du sexe atypique de *Mus minutoides*

Manuscrit 1: “XY females do better than the XX in the African Pygmy Mouse”

Chez les mammifères thériens, le déterminisme du sexe est très conservé, et toute déviation est normalement fortement contre sélectionnée à cause des problèmes de fertilité des individus possédant un complément en chromosomes sexuels non conventionnel. Les femelles XY par exemple sont généralement stériles, à cause de forts coûts reproductifs liés à la présence du chromosome Y et d'un X en copie unique (perte d'embryons YY, problèmes de méiose etc. ; Vernet et al., 2014). Dans ces conditions, un chromosome féminisant tel que le X* de *Mus minutoides* ne devrait pas pouvoir se maintenir, suggérant que des mécanismes évolutifs pour compenser la perte de fertilité doivent exister. Des données concernant la reproduction des trois types de femelles au sein de notre élevage ont été récoltées pendant presque trois ans, au cours desquels presque 500 portées (pour plus de 1500 petits) ont vu le jour. Ces données ont été utilisées pour comparer des traits tels que la proportion de femelles se reproduisant, l'âge à la première portée, les intervalles entre deux portées, et leur taille.

Les résultats mettent en évidence que les femelles X*Y chez la souris naine africaine ne sont pas lésées par leur complément en chromosomes sexuels en ce qui concerne la reproduction. Elles se reproduisent même plus souvent, et engendrent plus de descendants au cours de leur vie : elles produisent leur première portée en moyenne 20 jours plus tôt que les femelles XX et XX*, ce qui est non-négligeable considérant que leur espérance de vie en milieu naturel est estimée à un an (Happold 2013), et malgré la perte supposée d'un quart de leurs embryons (YY), elles produisent des portées plus grandes que les femelles XX et XX* (presque un petit en plus par portée), grâce à un taux d'ovulation plus fort. Ces résultats suggèrent que le maintien du chromosome X* chez *Mus minutoides* pourrait être assuré par une meilleure fitness des femelles X*Y en milieu naturel. Cependant il est probable que ces femelles aient eu une fitness réduite lors de l'apparition du chromosome féminisant (comme c'est le cas pour les femelles XY chez la grande majorité des mammifères et notamment la souris domestique), et que leur avantage reproductif ai évolué graduellement avec l'accumulation de mutations bénéfiques pour les femelles sur le chromosome X*.

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XY FEMALES DO BETTER THAN THE XX IN THE AFRICAN PYGMY MOUSE, *MUS MINUTOIDES*

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All therian mammals have a similar XY/XX sex-determination system except for a dozen species. The African pygmy mouse, *Mus minutoides*, harbors an unconventional system in which all males are XY, and there are three types of females: the usual XX but also XX* and X*Y ones (the asterisk designates a sex-reversal mutation on the X chromosome). The long-term evolution of such a system is a paradox, because X*Y females are expected to face high reproductive costs (e.g., meiotic disruption and loss of unviable YY embryos), which should prevent invasion and maintenance of a sex-reversal mutation. Hence, mechanisms for compensating for the costs could have evolved in *M. minutoides*.

Data gathered from our laboratory colony revealed that X*Y females do compensate and even show enhanced reproductive performance in comparison to the XX and XX*; they produce significantly more offspring due to (i) a higher probability of breeding, (ii) an earlier first litter, and (iii) a larger litter size, linked to (iv) a greater ovulation rate. These findings confirm that rare conditions are needed for an atypical sex-determination mechanism to evolve in mammals, and provide valuable insight into understanding modifications of systems with highly heteromorphic sex chromosomes.

KEY WORDS: **Breeding performance, life-history traits, sex chromosome evolution, sex-determination system, sex-reversed females, X* chromosome.**

Sex determination is a fundamental process of all sexual organisms. One could expect such an essential developmental process to be extremely conserved but the mechanisms involved are strikingly diverse (Bull 1983). Even in vertebrates, sex is determined by many strategies implying either genetic or environmental influences (or a combination of both). This diversity is observed at all taxonomic scales and the sex-determination system (SDS) often varies among closely related species. For example, fish or lizards have a great range of SDS, and within a same genus species can harbor either environmental or genetic sex determination (Devlin and Nagahama 2002). Diversity can even be found at a finer scale, as in the wrinkled frog *Rana rugosa*, in which sex is determined by sex chromosomes, but with different female heterogametic (ZZ/ZW) and male heterogametic (XX/XY) systems that evolved independently in different populations (Ogata et al. 2008; Uno et al. 2008). In contrast, some lineages have highly conserved SDS, such as snakes, birds, and mammals in which sex chromosomes are ancient. In particular, therian mammals (placentals and marsupials) possess a male heterogametic system that arose at least 148 million years ago (Mya; Veyrunes et al. 2008). Despite the apparent ubiquity and stability of this system, a dozen species are known to escape from the rule and harbor unusual SDS. For example, in the spiny rat *Tokudaia osimensis* and in the mole vole *Ellobius lutescens*, both males and females are X0, males having lost their Y chromosome and the mammalian sex-determining gene *Sry* with it (Soullier et al. 1998; Just et al. 2007; Kuroiwa et al. 2010). On the contrary, in the South American grass mice *Akodon* sp., the wood lemming *Myopus schisticolor*, and the arctic lemming *Dicrostonyx torquatus*, females are either XX or XY like males (Fredga et al. 1976; Fredga and Bulmer 1988; Bianchi 2002; Ortiz et al. 2009).

The most recently described unusual SDS is found in a close relative of the house mouse, the African pygmy mouse *Mus minutoides*, which also harbors high proportions of XY females (Veyrunes et al. 2010). Molecular and karyological analyses revealed that the sex-reversal mutation does not involve the male-determining *Sry* gene nor any other Y-linked genes, but rather the X chromosome. Indeed two different X chromosomes were identified by G-banding, varying in size and banding pattern: the ancestral X and a rearranged one, named X*, carrying the still unknown mutation responsible for the feminization of X*Y individuals. The comparison of phenotypic sex and genotypic sex (established from karyotypes) revealed the existence of three types of females: XX, XX*, and X*Y; whereas all males are XY (Veyrunes et al. 2010) and cytogenetics analyses suggest that the X* stopped recombining over a large region (unpubl. data). Sex-reversed females have been found from Southern up to Western Africa, and the use of molecular dating in a phylogenetic framework revealed that the system evolved at least 0.9 Mya (Veyrunes et al. 2013).

Evolution of such a system represents a paradox. Indeed, SDS with highly differentiated sex chromosomes are referred to as evolutionary traps (Pokorná and Kratochvíl 2009). Any deviation is usually prevented by the constraints imposed by the peculiar features of the sex chromosomes: accumulation of sexually antagonistic alleles and gene dosage compensation for example, linked to the divergence in sequence and gene content between the X and Y (Marin and Baker 1998; Marshall Graves 2006). Indeed, sex reversal in mammals, including pathological cases in human, often generates sterile individuals (for a review, see Vaiman and Pailhoux 2000). In laboratory strain mice, most XY females are sterile, due to meiotic defects leading to oocyte and embryo loss (Villemure et al. 2007; Alton et al. 2008; Lavery et al. 2011; Xu et al. 2012) and for those XY females that manage to bypass the constraints and pass meiosis, fertility is still greatly reduced compared to XX females, as 25% of embryos produced are unviable YY embryos.

How to explain the evolution and maintenance of the unusual SDS found in some mammals in these conditions? Several verbal and mathematical models have pointed out that transitions in SDS can be favored by different factors (reviewed in van Doorn 2014). Concerning the peculiar case of the feminizing X* chromosome, its maintenance would obviously be facilitated if X*Y females could avoid the fertility loss inherent to XY females in other mammals. Interestingly, in the other species with similar SDS to *M. minutoides* (see above), X*Y females have been described as having either an increased ovulation rate or a greater reproductive output (or both) compared to XX females (Gileva et al. 1982; Fredga and Bulmer 1988; Espinosa and Vitullo 1996). However, empirical data remain scarce.

Do X*Y females in *M. minutoides* escape the fate of most sex-reversed females in mammals, and avoid fertility loss? In this study, we used laboratory-reared animals to investigate differences in reproductive performance between the three types of females, and detail the contribution of several life-history traits to global reproductive output. Results show that X*Y females do not suffer from a reduced fertility and even have a significantly higher reproductive output compared to the XX and XX*. These results help understanding how such a peculiar system could be maintained for almost a million years in the African pygmy mouse and support that extremely rare conditions are necessary to allow deviation from a SDS with highly differentiated sex chromosomes.

Material and Methods

BREEDING COLONY

A breeding colony of *M. minutoides* was established in June 2010 from 13 wild-caught animals (eight females and five males) trapped in Caledon Nature Reserve, South Africa (29°54′ S, 26°51′ E). Very little is known about the ecology and life history of the African pygmy mouse and the few data available about its reproduction suggest a monogamous mating system (Skinner and Chimimka 2005), so mice were isolated in pairs in environmentally enriched terrariums. They were provided with ad libitum food and water, and light regime was set to 15:9 h (light:dark). Karyotypes of founder females were assessed based on a noninvasive fibroblast cell culture from tail-tip biopsy. Among the eight females, four were X*Y, three XX*, and one XX. Pairs were checked every two days to monitor dates of parturition and size of litters. Pups were sexed at weaning (25–28 days) by checking anogenital distance. Some of the offspring were paired for breeding purpose while others were involved in various experiments. To ensure a fast turnover in the colony, mates were replaced by others if no litter was produced after a six-month contact. As the whole pedigree is known (data archived online), inbreeding was limited by avoiding crosses between too closely related individuals (mean inbreeding coefficient: 0.054 ± 0.051). The genotype of females was systematically assessed by karyotyping and/or PCR amplification of the Y-specific *Sry* gene (Veyrunes et al. 2010). Karyotyping confirmed that XX females produce XY male and XX female offspring only, XX* produce males and the three types of females, and X*Y females give birth to XY sons, XX* and X*Y daughters, and are expected to produce 25% of nonviable YY embryos.

BREEDING PERFORMANCE

Data on breeding performance (e.g., litter size, date of birth) were collected for almost three years during which nearly 500 litters were produced (over 1500 pups). Due to constraints in the breeding setup and the need to use some females for other experiments,

we could not monitor the reproduction of every female during their whole life. Instead of lifetime reproductive success (the total number of offspring produced over an individual lifetime, see Clutton-Brock 1988), which would represent a key component of the fitness of each genotype, we compared the three types of females by estimating the overall number of offspring produced over the time the female was monitored. This parameter was estimated by integrating all females monitored regardless of time spent with a mate. As the overall number of offspring comprises many underlying components, to decipher potential differences, life-history traits involved in reproduction were subsequently measured: (i) proportion of breeding females, (ii) age at first litter, (iii) interbirth intervals, (iv) mean litter size, and (v) ovulation rate.

The proportion of breeding females was measured after six months of contact, all females that died/were sacrificed before this period and did not have at least one litter were excluded. The age at first litter was assessed for the three genotypes, knowing that the average age at contact (AAC) was 68.1 $\pm$ 40.3 days and that sexual maturity in *M. minutoides* starts at 6–8 weeks. The average litter size was measured at birth, which also reflects the litter size at weaning as preweaning mortality was extremely low in the laboratory colony. Finally, ovulation rate, measured as the number of corpora lutea at the surface of ovaries (see Fig. S1), was estimated at mid gestation (10–16 days post coitum) allowing to distinguish the corpora lutea resulting from follicles of the last ovulation cycle (that gave rise to the pregnancy) from older ones that may persist for several cycles (MacDowell 1924; Deanesly 1930). Ovulation rate was assessed for XX* and X*Y females only, as XX females that reproduced were too rare in the colony. Females were sacrificed by CO_2 inhalation. Sample sizes are indicated in figures' legends or Results section.

STATISTICS

All statistical analyses were performed using R (R Development Core Team 2013). In studies with inbred organisms, two related individuals are more likely to have similar traits than two individuals chosen randomly and are therefore not statistically independent. In this study, as the colony was founded by few mice, and even if crosses between close relatives were avoided, this nonindependence was clearly an issue. To deal with this problem, when it was possible, a kinship matrix built using the pedigree was incorporated in the models (Pinheiro and Bates 2000).

The distribution of the overall number of offspring did not follow a classical Poisson distribution as it showed a strong excess of zeros. From the different models that can deal with zero-inflated distributions of count data (Miller 2007), a Hurdle model with negative binomial distribution provided the best fit for analysis. Observation time was set as a covariate and the significance of the genotype on the overall number of offspring was assessed by comparing the fit of the model with or without genotype using Akaike information criterion (AIC). As available software allowing analysis of zero-inflated Poisson data have various analytical limitation, the kinship matrix was not taken into account. However, as the pedigree is known, it was possible to trace the origin of the X* chromosome (at least the nonrecombining region) of all XX* and X*Y females and assess whether (i) each X* copy has the same effect on breeding performance by adding the X* identity as a factor, and (ii) all different X* copies show the same trends using a genotype $\times$ X*interaction to the Hurdle model. As the X* identity and genotype $\times$ X* interaction have no influence on overall offspring number, we decided to not include them in the following models (analyzing the traits underlying the overall offspring number) as they limit comparisons to XX* and X*Y females only.

Concerning these underlying life-history traits, the nonindependence of females was taken into account in a way similar to how spatial autocorrelation is dealt with by using mixed models with an autocorrelated random effect (female identity) and a correlation matrix (the kinship matrix). Note that the random effect is necessary to implement the matrix despite there are no multiple measures for each female. The proportion of breeding females (binary data) was analyzed with a generalized linear mixed model (GLMM) using a model developed by Rousset and Ferdy (2014) specifically for GLMMs with autocorrelated random effects. AAC was included as a linear covariate and statistical inference was made using likelihood ratio test (LRT) as recommended by the authors. Mean litter size, the logarithm of age at first litter, the logarithm of mean interbirth interval, and ovulation rate were analyzed with linear mixed models. In all four models except the latter, AAC was also included as a covariate. For the ovulation rate model, AAC was not available and we used female age instead. Finally, for the mean litter size model an extra covariate was added: the age of female at first litter. Model simplification was made using AIC (Table S1). When the best model predicted a significant effect of genotype, Tukey's HSD test (Sokal and Rohlf 1995) was used to test post hoc differences between the three pairs of genotypes (XX vs. XX*, XX vs. X*Y, and XX* vs. X*Y). This test being inappropriate to compare genotypes after a Hurdle model or a GLMM, for the overall number of offspring and proportion of breeding females models, another method was used: to test differences between a pair of genotypes, LRTs between the best model and a model in which females of the two genotypes of interest are grouped in one same level were compared. A *P*-value inferior to the significance level (0.05) means the two levels paired are significantly different.

Table 1. Comparisons of pairs of genotype for overall number of offspring and proportion of breeding females using likelihood ratio tests (1 df) between the best model and a model in which two levels of the genotype variable are grouped. Significant values are shown in bold.

Life-history traits	XX versus XX*	XX versus X*Y	XX* versus X*Y
Overall number of offspring	$\chi^2 = 0.40$, $P = 0.527$	$\chi^2 = 7.80$, $\mathbf{P = 0.005}$	$\chi^2 = 12.00$, $\mathbf{P < 0.001}$
Proportion of breeding females	$\chi^2 = 0.91$, $P = 0.340$	$\chi^2 = 4.44$, $\mathbf{P = 0.035}$	$\chi^2 = 14.41$, $\mathbf{P < 0.001}$

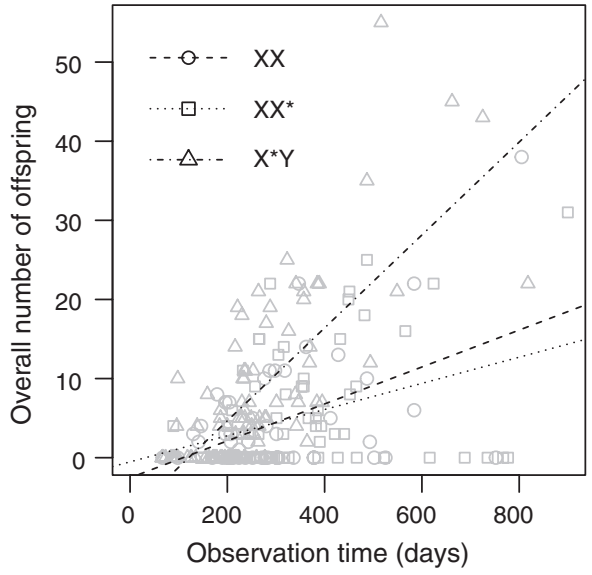

Figure 1. Overall number of offspring data and linear regression for the three types of females; sample sizes: XX: 48; XX*: 73; X*Y: 101.

Results

Model simplification of all models is presented in Table S1.

OVERALL NUMBER OF OFFSPRING

Figure 1 presents the data and regression lines for the three types of females. The Hurdle model with the best fit suggests a significant effect of the genotype on the overall progeny size ($F_{2,221} = 3.865$, P-value $= 0.005$), and the comparisons of the three genotypes suggest that XX and XX* females do not differ for this traits (P-value $= 0.527$), whereas differences are significant between X*Y versus XX and X*Y versus XX* (P-value $= 0.005$ and P-value < 0.001, respectively; Table 1). X*Y female produce more offspring than XX and XX* ones.

The integration of the X* identity in the model revealed that all X* copies show the same trend: there is no interaction between genotype and X* identity ($F_{4,165} = 0.14$, P-value $= 0.96$) and no effect of the X* identity on overall number of offspring produced ($F_{4,169} = 0.82$, P-value $= 0.51$).

PROPORTION OF BREEDING FEMALES

Respectively, 71% (25/35), 58% (38/65), and 89% (65/73) of XX, XX*, and X*Y females had at least one litter during their first six months of contact with a male. AAC has no impact on this trait ($F_{1,171} = 0.99$, P-value $= 0.32$), however there is a significant influence of genotype ($F_{2,170} = 4.040$, P-value $= 0.008$). Comparisons of the three genotypes suggest that XX and XX* females do not differ for this trait, whereas significantly more X*Y females succeeded in raising at least one litter (Table 1).

AGE AT FIRST LITTER

There is a highly significant effect of the AAC ($F_{1,122} = 128.1$, P-value < 0.001) and of the genotype ($F_{2,123} = 14.07$, P-value < 0.0001) on the age at first litter, but no interaction between AAC and genotype. Pairwise comparisons suggest that XX and XX* females have their first litter at the same age and that X*Y females have their first litter earlier than XX* females, but not significantly earlier than the XX ($P = 0.066$; Fig. 2A, Table 2). This value is marginally significant and rerunning the model while grouping XX and XX* females together showed that they have their first litter significantly later than the X*Y ones ($F_{1,124} = 29.11$, P-value < 0.001).

MEAN INTERBIRTH INTERVAL

Model simplification suggests that neither the AAC ($F_{1,73} = 2.75$, P-value $= 0.10$) nor the genotype ($F_{2,74} = 2.21$, P-value $= 0.12$) influence the mean interbirth interval (Fig. 2B, Table 2). The time between two litters is thus similar for the three types of females.

MEAN LITTER SIZE

Model simplification reveals the absence of an effect of the age at first litter ($F_{1,119} = 2.69$, P-value $= 0.1033$) and of the AAC ($F_{1,119} = 2.36$, P-value $= 0.13$), but a significant effect of the genotype ($F_{2,120} = 9.32$, P-value < 0.001) on the mean litter size. Multiple comparisons show that X*Y females have larger litters, with almost one extra pup on average compared to the XX and XX* females, the latter two producing litters of similar size (Fig. 2C, Table 2).

OVULATION RATE

Age of female ($F_{1,47} = 1.29$, P-value $= 0.26$) has no influence on the number of ova shed per cycle, but genotype does ($F_{1,48} = 15.98$, P-value < 0.001): X*Y females produce almost one and a half times more ova per cycle than XX* females do (Fig. 2D, Table 2).

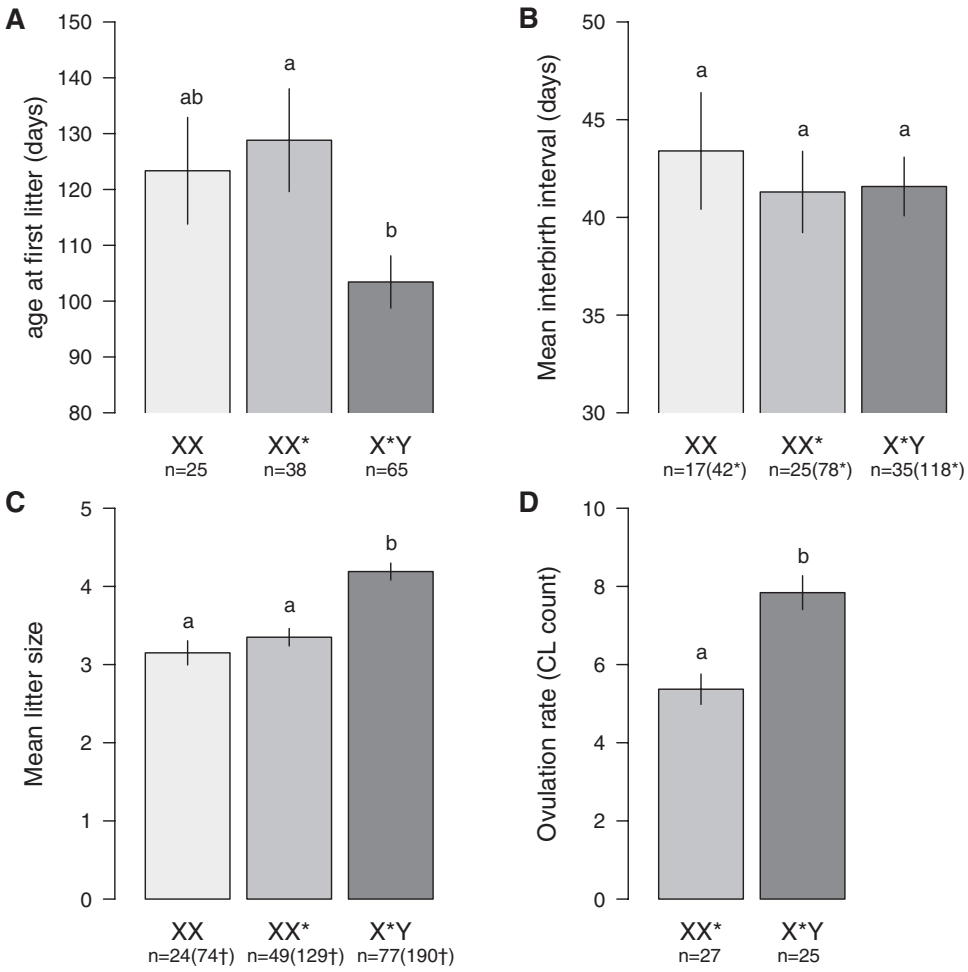

Figure 2. **Comparison of the three types of females in terms of life-history traits. Error bars represent standard error. Different letters above the bars indicate significant differences according to Tukey's HSD test or *F*-test (see Table 2). CL, corpora lutea, *n*, sample size (female number), *interval number, †litter number.**

Table 2. ***P*-values of pairwise comparisons using Tukey's HSD test for age at first litter and mean litter size at birth.**

Life-history traits	XX versus XX*	XX versus X*Y	XX* versus X*Y
Age at first litter (days)	$P = 0.90$	$P = 0.066$	$\mathbf{P = 0.008}$
Mean interbirth interval (days)	n/a	n/a	n/a
Mean litter size at birth	0.09	$\mathbf{P < 0.001}$	$\mathbf{P = 0.01}$
Ovulation rate (corpora lutea count)	–	–	$\mathbf{P < 0.001}$

Tukey's test is irrelevant for the average interbirth interval as genotype has no effect on this life-history trait, and also for ovulation rate as there are only two types of females; the *P*-value presented here comes from the *F*-test (see text). Significant values are shown in bold; n/a, not applicable.

Discussion

THE GREATER BREEDING PERFORMANCE OF X*Y FEMALES AND THE UNDERLYING MECHANISMS

The comparative analysis of the breeding performance of *M. minutoides* XX, XX*, and X*Y females clearly revealed a higher reproductive output of the X*Y. In the laboratory conditions, the overall number of offspring of these females was found to be higher than that of XX and XX* females (Fig. 1). To assert the proximal causes of this difference, several life-history traits linked to reproduction were studied and found to be involved: (i) a higher probability of breeding, (ii) an earlier first litter, and (iii) bigger litter size, related to (iv) a higher ovulation rate (Fig. 2).

Unlike laboratory mice, which are prolific breeders in captivity, in *M. minutoides* it could take up to several months for females

to have their first litter. Some females did not breed even after six months with a mate. Interestingly, there were significantly more X*Y females that bred at least once (89%) compared with XX and XX* females (71% and 59%, respectively; Table 1). Moreover, among the females that did breed, the sex-reversed females had their first litter in average 20 days earlier than the two other types of females (Fig. 2A, Table 2). A genotype-dependent physiological response may explain these differences. Indeed, XX and XX* females may have a lower fertilization success relatively to the X*Y. However, the sex chromosome complement has no influence on interbirth intervals (Fig. 2B), suggesting that once mice of a pair have accepted each other, they reproduce at the same rate regardless of the female's genotype, and thus, does not support the hypothesis of a lower fertilization success of XX and XX* females. Differences in terms of behavior could therefore be considered: males could express a preference, being eager or reluctant to mate with a female of a particular genotype, or females could have alternative reproductive strategies depending on their genotype. Indeed, differences in terms of behavior that could impact sexual interactions (aggressiveness, social interactions, stress . . .) have been described between XX and sex-reversed XY females in laboratory mice (Gatewood et al. 2006; McPhie-Lalmansingh et al. 2008). Further analyses implying mate choice experiments coupled with hormonal assays are thus required to elucidate this striking pattern.

In addition to a greater proportion of breeding females and earlier litters, larger litter size in X*Y females is all the more surprising: despite the loss of the YY embryos, they tend to produce almost one more pup per litter than the XX and XX* (Fig. 2C, Table 2). These differences can be explained by the higher ovulation rate, X*Y females producing almost one and a half times as many ova per cycle than the XX* females (Fig. 2D, Table 2).

The earlier first litter and higher ovulation rate of X*Y females suggest that they might undergo a shift in reproductive life span because of a faster depletion of the egg pool. Unfortunately, the age of mothers at last parturition was not monitored in this study. Therefore, X*Y females might not have a higher lifetime reproductive success, and differences we found in terms of overall number of offspring might not reflect actual fitness differences in the wild. However, some of the wild-caught X*Y females survived for over two years in captivity and were still breeding, going against a premature end of reproductive life. Moreover, in the wild, survival rates are low and few individuals live up to one year (Monadjem 2013) and populations undergo drastic fluctuations in density (Britton-Davidian et al. 2012 and references therein). These arguments suggest that X*Y females are likely to experience a higher fitness in the wild, and early fecundity that is favored in rapidly increasing populations (Caswell 1982), might add to the success of the X*.

EVOLUTION AND STABILITY OF THE SYSTEM

Interestingly, X*Y females found in some *Akodon* mice and lemmings do not suffer from meiotic defects and loss of embryos that are usually associated with sex reversal in mammals either. X*Y females in the South American grass mouse *A. azarae* have a longer reproductive life span and a higher rate of preimplantation embryonic development (Espinosa and Vitullo 1996, 2001), and in the arctic and wood lemmings *D. torquatus* and *M. schisticolor*, they have a greater ovulation rate (Fredga and Bulmer 1988; Fredga 1994). In addition, in the latter species, the Y chromosome of X*Y females is eliminated from the germ line by a unique mechanism of double nondisjunction in fetal ovaries preventing the formation of YY embryos (Fredga 1994). Hence, independently, different or similar mechanisms evolved to bypass the reproductive disadvantage of X*Y females, and this probably represents a crucial requirement to provide stability to such atypical SDS. This postulate is consistent with analytical findings showing that a feminizing X* chromosome can be maintained in a population only if it confers a selective advantage to the females bearing it (Bengtsson, pers. comm. 1977). So, invasion of the X* could have been triggered by an inversion carrying a feminizing factor together with genes conferring increased breeding performance. Alternatively, this unusual system could also have appeared despite a poor fertility of the sex-reversed females at first (which is likely given that the African pygmy mouse is a close relative of the house mouse, in which XY females are sterile or have a very poor fertility), their increased breeding performance evolving subsequently. Indeed, several mathematical models have shown that factors other than a fitness advantage of the mutation could explain modifications of SDS. According to these models, the invasion and maintenance of a new sex-determining mutation such as the X* could happen, for example, in circumstances favoring a female biased sex ratio, for example, under a regime of interdemic selection (Vuilleumier et al. 2007), to resist a selfish element causing meiotic drive (Kozielska et al. 2010), under sexually antagonistic selection (van Doorn and Kirkpatrick 2007, 2010) or under strong inbreeding, the latter being supported by empirical work on *M. schisticolor* (Maynard Smith and Stenseth 1978).

CONSEQUENCES OF THE EMERGENCE OF THE X* ON THE EVOLUTION OF THE SEX CHROMOSOMES, WHAT MAKES X*Y FEMALES BETTER?

Sex chromosomes have a very particular gene content (Vallender and Lahn 2004; Marshall Graves 2006). In mammals, the X harbors an excess of genes with gametogenesis and reproductive functions compared to autosomes, and the Y is gene poor and has a highly male biased set of genes. Multiple forces drive the evolution of these peculiar gene contents, for example, lack of recombination, responsible for degradation of heterogametic chromosomes,

and hemizygous exposure and sex-biased transmission, which both influence the accumulation or loss of some types of sexually antagonistic alleles (e.g., Rice 1984; Charlesworth and Charlesworth 2000; Ellegren and Parsch 2007). The way this complex set of evolutionary forces acts on the sex chromosomes must have been disturbed when the X* chromosome appeared, changing the evolutionary trajectories of all three sex chromosomes. For example, effective sizes and transmission patterns of the X and Y have been modified, changing the pressures on sexually antagonistic genes. As for the X*, with the acquisition of a female uniparental transmission, theory predicts it will evolve like a heterogametic chromosome, becoming prone to degeneration but more interestingly to the accumulation of female beneficial genes and silencing/pseudogenization of male beneficial genes, which could result in making the X* chromosome a "better female chromosome" than the X.

But has the X* in *M. minutoides* appeared long enough ago to leave enough time for the sex chromosomes to evolve in the way predicted by theory? Recent studies support the fact that young sex chromosomes can evolve quickly: the 2 Mya neo-sex chromosomes of the threespine stickleback *Gasterosteus aculeatus* show traces of genetic differentiation (Natri et al. 2013) and the 1 Mya old neo-Y chromosome of *Drosophila miranda* shows degeneration and masculinization signs (Bachtrog et al. 2008; Zhou and Bachtrog 2012). Despite the obvious difference between *Drosophila* and mice in terms of generation time, it would not be surprising that the gene content of the X and X* have started diverging, especially considering that recombination probably stopped over a large region of the X* around 0.9 Mya (inferred from BAC-mapping experiments; unpubl. data).

Linking theory and these empirical observations to the fact that X*Y females have greater breeding performance, suggests that a peculiar feature of the X*Y complement is responsible for this advantage. Several hypotheses are discussed here. First, in regard to the theory, it is tempting to attribute the greater reproductive outcome of X*Y females to the X*. As it is expected to accumulate female-beneficial genes (see above), it could have evolved an intrinsic advantage relatively to the X chromosome. However XX* females also bear this chromosome but have identical breeding performance to XX females. As in female mammals one of the two X chromosomes becomes transcriptionally silent in each cell (Chow and Heard 2009), we could expect XX* females to have an intermediate breeding success, unless a mechanism favoring the inactivation of the X* evolved, or if a deleterious dominant X-linked allele that escapes inactivation (Carrel and Willard 2005; Yang et al. 2010) spread to fixation. Second, there could be an influence of the Y chromosome or an interaction between the X* and the Y, this would explain why XX* females are similar to XX females. However, it is harder to conceive that a chromosome that is so extremely specialized in male function could be advantageous for females unless repeated translocations of genes involved in female reproduction occurred. Finally, another feature that sets X*Y apart from XX and XX* females is the number of X chromosome harbored. It was recently shown using laboratory mice with various sex chromosome complements (e.g., XX, X0, and XY females) that the number of X chromosomes influences the expression of hundreds of autosomal genes (Wijchers et al. 2010). Despite a primordial role of the sex chromosomes in sexual traits, the autosomes harbor the majority of genes with sex-biased expression (Mank 2009). The phenotypic differences between the females could therefore be due to differential expression of autosomal genes sensitive to the number of X.

Conclusion

In *M. minutoides*, X*Y females show enhanced breeding performance in comparison to the XX and the XX*. This remarkable finding helps understanding how such an unusual SDS can evolve and be maintained in a mammal species. Many pieces of the puzzle are still missing to fully understand the evolution of this peculiar system, but this study highlights that other approaches (e.g., behavior, mathematical modeling, next generation sequencing) will be useful to further decipher its evolution. The uniqueness of this system also lies in the fact that the X* evolved as a third sex chromosome that has acquired a female-limited transmission and no longer recombines (to which extent we still not know). So more broadly, this system offers a great opportunity to study the early stages of the evolution of a novel sex chromosome: from the first steps of loss of recombination to the accumulation of sex-related genes on a heterogametic sex chromosome, processes that are still poorly understood due to the scarcity of adequate biological models.

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DATA ARCHIVING

The doi for our data is 10.5061/dryad.j18g0.

LITERATURE CITED

Alton, M., M. P. Lau, M. Villemure, and T. Taketo. 2008. The behavior of the X- and Y-chromosomes in the oocyte during meiotic prophase in the B6.Y(TIR) sex-reversed mouse ovary. Reproduction 135:241–252.

Bachtrog, D., E. Hom, K. M. Wong, X. Maside, and P. de Jong. 2008. Genomic degradation of a young Y chromosome in *Drosophila miranda*. Genome Biol. 9:R30.

Bianchi, N. O. 2002. *Akodon sex* reversed females: the never ending story. Cytogenet. Genome Res. 96:497–501.

Britton-Davidian, J., T. J. Robinson, and F. Veyrunes. 2012. Systematics and evolution of the African pygmy mice, subgenus *Nannomys*: a review. Acta Oecol. 42:41–49.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park, CA.

Carrel, L., and H. F. Willard. 2005. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434: 400–404.

Caswell, H. 1982. Life history theory and the equilibrium status of populations. Am. Nat. 120:317–339.

Charlesworth, B., and D. Charlesworth. 2000. The degeneration of Y chromosomes. Phil. Trans. R. Soc. Lond. B 355:1563–1572.

Chow, J., and E. Heard. 2009. X inactivation and the complexities of silencing a sex chromosome. Curr. Opin. Cell Biol. 21:359–366.

Clutton-Brock, T. H. 1988. Reproductive success. Studies of individual variation in contrasting breeding systems. University of Chicago Press, Chicago.

Deanesly, R. 1930. The corpora lutea of the mouse, with special reference to fat accumulation during the oestrous cycle. Proc. R. Soc. Lond. B 106:578–595.

Devlin, R. H., and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191–364.

Ellegren, H., and J. Parsch. 2007. The evolution of sex-biased genes and sex-biased gene expression. Nat. Rev. Genet. 8:689–698.

Espinosa, M. B., and A. D. Vitullo. 1996. Offspring sex-ratio and reproductive performance in heterogametic females of the South American field mouse *Akodon azarae*. Hereditas 124:57–62.

———. 2001. Fast-developing preimplantation embryo progeny from heterogametic females in mammals. Zygote 9:289–292.

Fredga, K. 1994. Bizarre mammalian sex-determining mechanisms. *In* R. V Short and E. Balaban, eds. The differences between the sexes. Cambridge Univ. Press, New York.

Fredga, K., and M. Bulmer. 1988. Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. Philos. Trans. R. Soc. Lond. B 322:83–95.

Fredga, K., A. Gropp, H. Winking, and F. Frank. 1976. Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*. Nature 261:225–227.

Gatewood, J. D., A. Wills, S. Shetty, J. Xu, A. P. Arnold, P. S. Burgoyne, and E. F. Rissman. 2006. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. J. Neurosci. 26:2335–2342.

Gileva, E. A., I. E. Benenson, L. A. Konopistseva, V. F. Puchkov, and I. A. Makaranets. 1982. XO females in the varying lemming, *Dicrostonyx torquatus*: reproductive performance and its evolutionary significance. Evolution 36:601–609.

Just, W., A. Baumstark, A. Süss, A. Graphodatsky, W. Rens, N. Schäfer, I. Bakloushinskaya, H. Hameister, and W. Vogel. 2007. Ellobius lutescens: sex determination and sex chromosome. Sex. Dev. 1:211–221.

Kozielska, M., F. J. Weissing, L. W. Beukeboom, and I. Pen. 2010. Segregation distortion and the evolution of sex-determining mechanisms. Heredity 104:100–112.

Kuroiwa, A., Y. Ishiguchi, F. Yamada, A. Shintaro, and Y. Matsuda. 2010. The process of a Y-loss event in an XO/XO mammal, the Ryukyu spiny rat. Chromosoma 119:519–526.

Lavery, R., A. Lardenois, F. Ranc-Jianmotamedi, E. Pauper, E. P. Gregoire, C. Vigier, C. Moreilhon, M. Primig, and M.-C. Chaboissier. 2011. XY *Sox9* embryonic loss-of-function mouse mutants show complete sex reversal and produce partially fertile XY oocytes. Dev. Biol. 354:111–122.

MacDowell, E. 1924. A method of determining the prenatal mortality in a given pregnancy of a mouse without affecting its subsequent reproduction. Anat. Rec. 27:329–336.

Mank, J. E. 2009. Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. Am. Nat. 173:141–150.

Marin, I., and B. S. Baker. 1998. The evolutionary dynamics of sex determination. Science 281:1990–1994.

Marshall Graves, J. A. 2006. Sex chromosome specialization and degeneration in mammals. Cell 124:901–914.

Maynard Smith, J., and N. C. Stenseth. 1978. On the evolutionary stability of the female-biased sex-ratio in the wood lemming (*Myopus schisticolor*): the effect of inbreeding. Heredity 41:205–214.

McPhie-Lalmansingh, A., L. Tejada, J. Weaver, and E. Rissman. 2008. Sex chromosome complement affects social interactions in mice. Horm. Behav. 54:565–570.

Miller, J. 2007. Comparing Poisson, Hurdle, and ZIP model fit under varying degrees of skew and zero-inflation. University of Florida, Gainesville, FL.

Monadjem, A. 2013. *Mus minutoides* Tiny Pygmy Mouse. *In* D. C. D. Happold, ed. Mammals of Africa. Vol. 3: Rodents, Hares and Rabbits. University of California Press, Berkeley, CA.

Natri, H. M., T. Shikano, and J. Merilä. 2013. Progressive recombination suppression and differentiation in recently evolved neo-sex chromosomes. Mol. Biol. Evol. 30:1131–1144.

Ogata, M., Y. Hasegawa, H. Ohtani, M. Mineyama, and I. Miura. 2008. The ZZ/ZW sex-determining mechanism originated twice and independently during evolution of the frog, *Rana rugosa*. Heredity 100:92–99.

Ortiz, M. I., E. Pinna-Senn, G. Dalmasso, and J. A. Lisanti. 2009. Chromosomal aspects and inheritance of the XY female condition in *Akodon azarae* (Rodentia, Sigmodontinae). Mamm. Biol. 74:125–129.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-Plus. Springer, New York.

Pokorná, M., and L. Kratochvíl. 2009. Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? Zool. J. Linn. Soc. Lond. 156:168–183.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at http://www.R-project.org/.

Rice, W. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.

Rousset, F., and J.-B. Ferdy. 2014. Testing environmental and genetic effects in the presence of spatial autocorrelation. Ecography: *In press*. doi: 10.1111/ecog.00566.

Skinner, J. D., and C. T. Chimimka. 2005. The mammals of the Southern African subregion. 3rd ed. Cambridge Univ. Press, New York.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman and Company, New York.

Soullier, S., C. Hanni, F. Catzeflis, P. Berta, and V. Laudet. 1998. Male sex determination in the spiny rat *Tokudaia osimensis* (Rodentia: Muridae) is not *Sry* dependent. Mamm. Genome 9:590–592.

Uno, Y., C. Nishida, Y. Oshima, S. Yokoyama, I. Miura, Y. Matsuda, and M. Nakamura. 2008. Comparative chromosome mapping of sex-linked genes and identification of sex chromosomal rearrangements in the Japanese wrinkled frog (*Rana rugosa*, Ranidae) with ZW and XY sex chromosome systems. Chromosome Res. 16:637–647.

Vaiman, D., and E. Pailhoux. 2000. Mammalian sex reversal and intersexuality: deciphering the sex-determination cascade. Trends Genet. 16:488–494.

Vallender, E. J., and B. T. Lahn. 2004. How mammalian sex chromosomes acquired their peculiar gene content. BioEssays 26:159–169.

Van Doorn, G. S. 2014. Evolutionary transitions between sex-determining mechanisms: a review of theory. Sex. Dev. 8:7–19.

Van Doorn, G. S., and M. Kirkpatrick. 2007. Turnover of sex chromosomes induced by sexual conflict. Nature 449:909–912.

———. 2010. Transitions between male and female heterogamety caused by sex-antagonistic selection. Genetics 186:629–645.

Veyrunes, F., P. D. Waters, P. Miethke, W. Rens, D. McMillan, A. E. Alsop, F. Grützner, J. E. Deakin, C. M. Whittington, K. Schatzkamer, et al. 2008. Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. Genome Res. 18:965–973.

Veyrunes, F., P. Chevret, J. Catalan, R. Castiglia, J. Watson, G. Dobigny, T. J. Robinson, and J. Britton-Davidian. 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. Lond. B 277:1049–1056.

Veyrunes, F., J. Perez, S. N. C. Paintsil, E. Fichet-Calvet, and J. Britton-Davidian. 2013. Insights into the evolutionary history of the X-linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked *Sry* gene. Sex. Dev. 7:244–252.

Villemure, M., H.-Y. Chen, M. Kurokawa, R. A. Fissore, and T. Taketo. 2007. The presence of X- and Y-chromosomes in oocytes leads to impairment in the progression of the second meiotic division. Dev. Biol. 301:1–13.

Vuilleumier, S., R. Lande, J. J. M. Van Alphen, and O. Seehausen. 2007. Invasion and fixation of sex-reversal genes. J. Evol. Biol. 20:913–920.

Wijchers, P. J., C. Yandim, E. Panousopoulou, M. Ahmad, N. Harker, A. Saveliev, P. S. Burgoyne, and R. Festenstein. 2010. Sexual dimorphism in mammalian autosomal gene regulation is determined not only by *Sry* but by sex chromosome complement as well. Dev. Cell 19:477–484.

Xu, B., Y. Obata, F. Cao, and T. Taketo. 2012. The presence of the Y-chromosome, not the absence of the second X-chromosome, alters the mRNA levels stored in the fully grown XY mouse oocyte. PLoS One 7:e40481.

Yang, F., T. Babak, J. Shendure, and C. M. Disteche. 2010. Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res. 20:614–622.

Zhou, Q., and D. Bachtrog. 2012. Sex-specific adaptation drives early sex chromosome evolution in *Drosophila*. Science 337:341–345.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Left ovary of an XX* female on which there are three corpora lutea (CL).

Table S1. Details of model simplification for each model, using (i) AIC for the overall number of offspring (a), age at first litter (c), mean interbirth interval (d), mean litter size (e), and ovulation rate (f). We consider that a difference in AIC of 0-2 means that the models have similar fits; (ii) likelihood ratio tests for the proportion of breeding females (b).

Manuscrit 2: “Genomic conflicts and the evolution of an unusual sex determination system in the African pygmy mouse”

Lors de l'analyse des données de reproduction des trois types de femelles (manuscrit 1), nous avons remarqué que les femelles semblaient produire plus de mâles que sous l'attendu de transmission aléatoire des chromosomes sexuels. L'analyse des sex-ratios dans les portées des trois types de femelles ont révélé que les femelles XX produisent 80% de mâles et les femelles XX* et X*Y respectivement 37% et 42% (contre 50%, 25% et 33% sous l'hypothèse de ségrégation mendélienne). En génotypant l'ensemble des individus issus des croisements, il a été mis en évidence que la production d'un excès de mâles est liée à une distorsion du ratio de transmission des chromosomes sexuels des mâles, et de manière surprenante, la force et le sens de la distorsion sont dépendants du génotype de la femelle (la distorsion est dite « conditionnelle »). Accouplé à une femelle XX ou XX*, un mâle voit son chromosome Y transmis à 80% de sa progéniture, et accouplé à une femelle X*Y, c'est son chromosome X qui est transmis majoritairement (64%).

Ces résultats ont été reliés à une hypothèse issue de la littérature visant à expliquer certaines transitions d'un système de déterminisme du sexe à un autre : une modification du mode de déterminisme du sexe pourrait survenir en réponse à un conflit génomique affectant le sex-ratio (Werren and Beukeboom 1998). Récemment, Kosielska et ses collaborateur (2010) ont développé un modèle théorique montrant qu'une mutation féminisante dans un système de déterminisme hétérogamétique pouvait envahir en réponse à un élément génétique égoïste biaisant la transmission du chromosome Y. Le lien a rapidement été fait avec nos résultats empiriques, et un ensemble de modèles de génétique des populations (prenant en compte l'ensemble des résultats issus de l'analyse de la reproduction des souris naines : meilleure fertilité des femelles X*Y et distorsions de transmission des chromosomes sexuels mâles) a été développé pour tester différents scénarios pour expliquer l'évolution du déterminisme du sexe atypique de Mus minutoides. Les modèles montrent (i) que le X* aurait bel et bien pu évolué en réponse à un distorteur de transmission du chromosome Y, mais (ii) que le(s) élément(s) génétique(s) biaisant le sex-ratio aurait également pu évolué suite à l'apparition du chromosome X*, à condition que les femelles X*Y aient déjà un meilleur succès reproducteur que les autres femelles. Ces modèles mathématiques nous ont également permis d'investiguer les conditions permettant l'évolution de la curieuse distorsion de transmission conditionnelle des chromosomes sexuels mâles chez cette espèce. Les résultats montrent que selon les différents scénarios évolutifs, différents compartiments du génome (chromosomes sexuels, autosomes) pourraient porter les éléments génétiques distorteurs, et que ce genre de système de déterminisme polygénique à l'air relativement tolérant envers les distorteurs de transmission des chromosomes sexuels.

Genomic conflicts and the evolution of an unusual sex determination system in the African pygmy mouse

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Abbreviations: SDS, Sex determination system; TDMSC, transmission distortion of male sex chromosomes

INTRODUCTION

Sex determination is an essential process for all sexually reproductive species with separate males and females. Quite surprisingly, the mechanisms involved in this process are extremely diverse (Bull 1983; Bachtrog et al. 2014; Beukeboom and Perrin 2014): sex can be determined by a wide variety of factors, ranging from genetic factors, e.g. the widespread XX/XY male heterogamety found in drosophila and therian mammals, to environmental cues, as in turtles or crocodiles which have a temperature-dependent sex determination. The increasing accumulation of data on the diversity of the sex determination system (SDS) harboured by many eukaryotes, and its recent integration in a phylogenetic framework have helped better understanding some aspects of the evolution of sex determination (e.g. Gamble et al. 2015; Pennell et al. 2015; Vicoso and Bachtrog 2015). However, the ultimate causes responsible for the shift from one system to another are still largely unclear, expect for a handful of cases (e.g. Roberts et al. 2009; Cordaux et al. 2011). Potential causes for transitions have nevertheless been thoroughly explored theoretically, and several evolutionary mechanisms have been proposed (see reviews in Sander van Doorn 2013, 2014; Beukeboom and Perrin 2014). Amongst the different hypotheses, one relies on the ability of emergent sex determiners to respond to selfish genetic elements that skew the transmission of sex chromosomes. So called sex chromosome drivers have been recognized as a major evolutionary force involved in the evolution of many fundamental aspects of sexual reproduction (Taylor & Ingvarsson, 2003), and were hypothesized to explain the evolution of unusual SDS found in several mammalian species. It was proposed that a driving X chromosome could have been responsible for the evolution of the X0/XY system found in *Microtus oregoni* (Charlesworth and Dempsey 2001), and that similar genomic conflicts could

have led to the emergence of XX intersexes (females with ovotests) in *Talpa occidentalis* (McVean and Hurst 1996). To clarify the conditions allowing a transition from one chromosomic SDS to another in response to selfish genetic elements biasing sex-ratio, a general theoretical framework was developed by Kozielska et al. (2010). It shows for example that in an XX/XY SDS, a Y chromosome drive creates a strong selection pressure favouring the evolution of a female sex determiner on the X.

Such a female sex determiner has been found on the X of the African pygmy mouse *Mus minutoides*, which was recently added to the short list of mammals with unusual sex determination (Veyrunes et al. 2010). *Mus minutoides* has a polygenic SDS: two independent sex determination genes segregate in natural populations: the regular mammalian male determiner *Sry* on the Y chromosome, and a still unknown dominant female determiner on the X chromosome, which led to the emergence of a third sex chromosome, named X*. The X* prevents the masculinization of X*Y embryos, so while all males are XY in this species, there are three types of females: the classic XX, the heterozygous XX* and the sex-reversed X*Y females. It was proposed that the X* is maintained in natural populations thanks to the greater reproductive performances of X*Y females (Saunders et al. 2014), in agreement with theoretical models by Bull and Charnov, who showed that a sex determining allele can invade if it provides a selective advantage to individuals bearing it, (1977). However, it is likely that when the mutation appeared (almost one million years ago; Veyrunes et al. 2013), X*Y females had a poor breeding success, like it is typically the case in sex-reversed mice (Mahadevaiah et al. 1993). Another mechanism could therefore have been at the origin of the spread of the feminising mutation: the X* could have evolved in response to the invasion of a selfish genetic element biasing sex-ratio towards males: most likely a transmission distorter of the Y chromosome.

To test this hypothesis, we analysed the transmission ratio of sex chromosomes in crosses involving the three types of females. Examination of the results revealed a very strong transmission distortion of male sex chromosomes, which surprisingly, is dependent on female genotype. Males transmit their Y more often in crosses with XX and XX* females and their X in crosses with X*Y females. Based on these results, and the previous ones showing that X*Y females have a greater breeding success than XX and XX* females, we developed a set of population genetics models (inspired by theoretical work by Bull & Bulmer (1981) aiming at understanding how XY females persist in natural populations in two species of lemmings), to (i) propose a scenario to explain the evolution of this

polygenic sex determination system in *Mus minutoides*, and (ii) better understand the evolution of this unprecedented sex chromosome transmission distortion in which males transmit their X or Y chromosome more often depending on female genotype.

RESULTS

Transmission ratio of male sex chromosomes depends on their female mate's genotype.

The expected sex-ratio in the progenies of the three types of females, and observed sex-ratio at weaning are shown in table 1. The proportion of males produced was significantly higher than the expected in the three types of crossings.

Female genotype	XX	XX*	X*Y
Expected sex-ratio	0.5	0.25	0.33
Observed sex-ratio (overall number of offspring)	0.79 +/- 0.13 (206)	0.37 +/- 0.17 (370)	0.42 +/- 0.14 (670)
Departure from expected sex-ratio (Binomial test)	p=<2.2e-16	p=1.967e-07	p=6.701e-05

Table 1. Expected *vs*. observed sex ratio in the progenies of the three types of females. The expected sex-ratio in the progenies of XX* and X*Y females is different from 0.5 because they produce viable offspring of respectively four genotypes (XX, XX*, X*Y and XY) and three genotypes (XX*, X*Y, XY).

To confirm that all females produce litters with biased sex-ratio, Hartigans' test of unimodality (Hartigan and Hartigan 1985) was used based on the average sex-ratio of the overall progeny of each female. The test failed to detect multimodality in the sex-ratio distributions of the three types of females (XX females D=0.088, p-value=0.087, XX* females D=0.075, p-value=0.071, X*Y females: D=0.053, p-value=0.23) suggesting that the genetic element(s) driving sex-ratio is (are) fixed.

It is straightforward that the skew in the sex-ratio of the progeny of XX females results from a transmission distortion of male sex chromosomes (TDMSC): There is an average of 79% of males in their progeny, meaning that males transmit their Y chromosome

to roughly 80% of their offspring (table 1). To know whether sex-ratio bias in the progenies of XX* and X*Y females is due to a skewed transmission of male or female sex chromosomes (or both), all of their offspring were typed (see methods) to measure the transmission ratio of sex chromosomes in the two sexes (table 2A). The transmission ratio of sex chromosomes in XX* and X*Y females was not significantly different from 50/50 (table 2B). On the other hand, males paired with XX* females see their Y transmitted to almost 80% of their progeny, (like males paired to XX females), and transmission ratio of sex chromosomes of males paired to X*Y females is also biased, but surprisingly, in the other direction. It is their X chromosome which is transmitted more often, the Y chromosome being transmitted to only 36% of their offspring. We call this TDMSC a "conditional" distortion, as it depends on the female's genotype.

A.

Female genotype		**XX**	**XX***		**X*Y**	
Sex chromosomes		X	X	X*	X*	Y
Males	X	43	37	52	248	283
	Y	163	135	146	139	†

B.

	transmission ratio	**p-value (binomial test)**
Males with XX females	**Y: 0.791**(95% CI: 0.730-0.845)	**<2.2e-16**
Males with XX* females	**Y: 0.760** (95% CI: 0.712-0.802)	**<2.2e-16**
Males with X*Y females[1]	**Y: 0.359** (95% CI: 0.311-0.409)	**3.287e-08**
XX* females	**X: 0.465** (95% CI: 0.413-0.517)	0.1936
X*Y females[1]	**X*: 0.467** (95% CI: 0.434-0.511)	0.14

Table 2. Transmission ratios of sex chromosomes in males and in XX* and X*Y females. A: results of the genotyping, number of each type of offspring in the progeny of the three types of crosses. B. Transmission ratios of sex chromosomes. [1]as X*Y females produce YY embryos, to determine the transmission ratio of sex chromosomes of males and females involved in these crosses, we compared the proportion of XX* *vs*. X*Y females (248 *vs*. 139) and XX* females *vs*. XY males (248 *vs*. 283) respectively.

Theoretical analyses

We developed a set of population genetics models to investigate how a standard XX/XY sex determination system could evolve into a polygenic (XX-XX*-X*Y/XY) SDS with conditional TDMSC (see methods and appendix 1 at the end of the manuscript). In the rest of the paper, the relative fitness of X*Y females is denoted as *w* (XX and XX* females showing identic reproductive performances (Saunders et al. 2014), their fitness is set to 1) and *k* and *k** denote the transmission ratio of male Y chromosome in crosses with respectively XX or XX* females and X*Y females.

Two paths are considered, that relate to two types of triggers for the initial invasion of the X*: (i) either the X* emerged in response to the evolution of a transmission distorter (Kozielska et al. 2010), or (ii) the invasion of the X* was triggered by a fitness advantage of its bearers (Bull and Charnov 1977), here the X*Y females. In that case the distorter(s) could have evolved subsequently.

The scenarios

To study the transition in sex determination in *Mus minutoides* based on these two hypotheses (response to a transmission distorter and fitness advantage), different scenarios are considered (Figure 1), involving several steps (two or three).

Scenarios A and B relate to the first hypothesis. A common initial step (**step 1a** in the figure 1) is the invasion of a driving Y chromosome. In scenario A (continuous blue arrows), we consider the evolution of an X* that has no effect on transmission of male sex chromosomes (**step 2a**), which leads to a system with polygenic sex determination in which male Y chromosomes are transmitted preferentially in the three types of crosses (a likely intermediate stage before the evolution of the conditional TDMSC), followed by the invasion of a conditional TDMSC modifying distortion specifically in crosses with X*Y females (**step 3**). As we don't know which compartment of the genome harbours the mutation, and that different genomic compartments have conflicting interests when it comes to the transmission ratio of sex chromosomes, we analyse three possibilities: in our models, the mutation is harboured either by an autosome, the Y or the X*. In scenario B (blue dashed arrows), we investigate the conditions allowing the invasion of a mutation that causes both the sex reversal phenotype and the modification of distortion in X*Y females (**step 1b**).

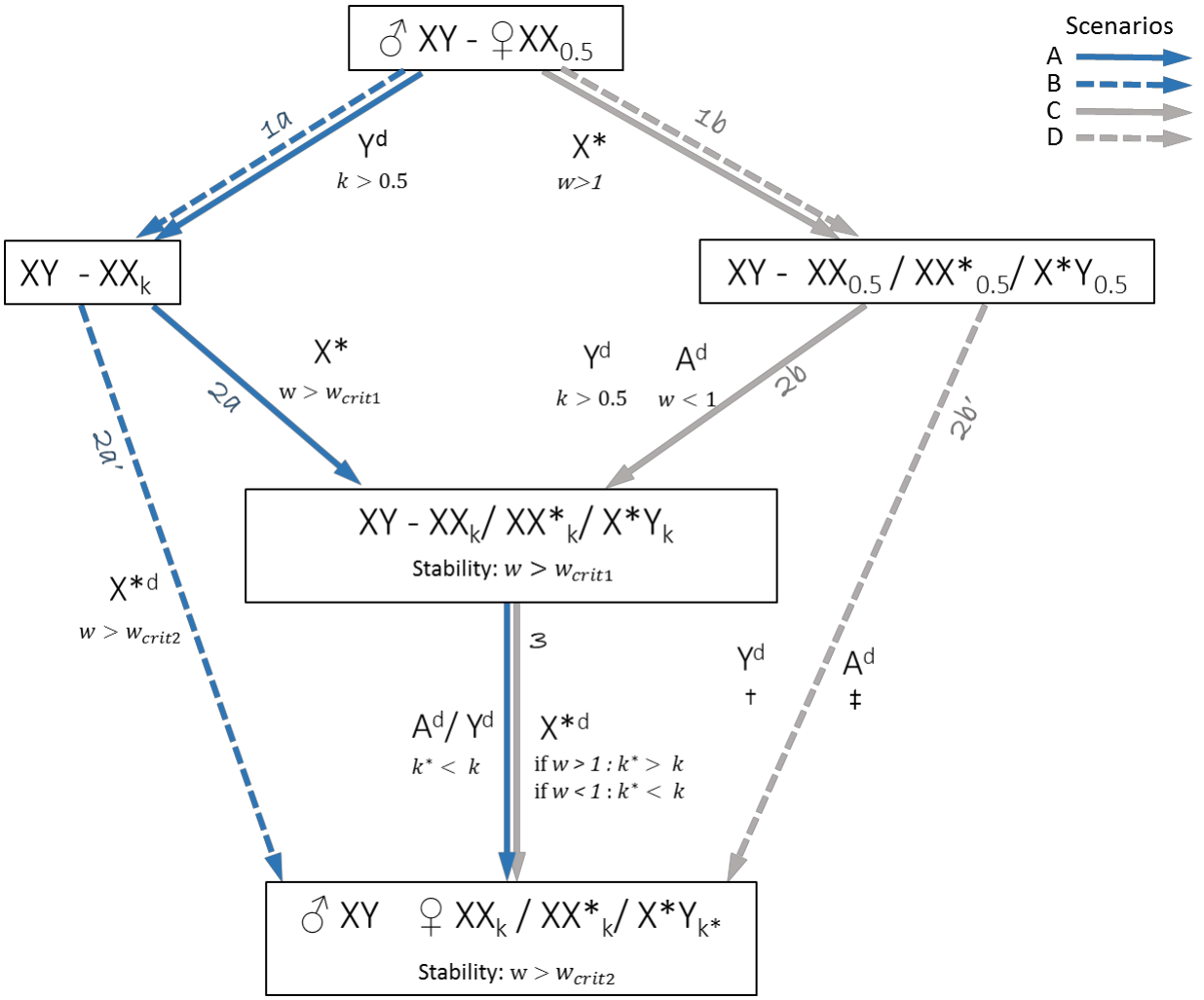

Figure 1. Paths to the evolution of the unusual sex determination of *Mus minutoides*.

Each box represents a stable state in which sex determination is given (either standard male heterogamety, or a polygenic X/X*/Y sex determination system). The subscripts (0.5, k, k*) refer to the transmission ratio of male sex chromosomes in each cross. And the condition for stability of the state is given under the previous information. Arrows represent steps (1a, 2a…) leading from one state to another. The type/colour of the arrow denote the different scenarios described in the text. At each arrow corresponds an invasion analysis, and next to the arrow is (i) the chromosome that harbours the mutation considered (A for autosome), the superscript informs on the type of mutation (*: feminising mutation, d: transmission distorter), (ii) the condition for invasion.

$\boldsymbol{w_{crit1}} = \frac{1-k}{k}$, $\boldsymbol{w_{crit2}} = \frac{1-k}{k} * \frac{1-k}{\frac{1}{2}-k^*\left(1-\frac{1}{2k}\right)}$,

†: $k \geq \frac{1}{2}\frac{w^2+1}{2w^2-w+1}$ and $k^* \leq \frac{1}{2}\frac{4kw^2-w^2+2k-2kw-1}{2w^2-2kw}$ or $w < 1+\sqrt{2}$ and $k \geq \frac{1}{2}\frac{w+1}{w^2+1}$

‡: see text and appendix

Scenarios C and D relate to the fitness advantage hypothesis. The common first step is the invasion of an X^* chromosome (1b), made possible by the fitness advantage of X^*Y females. In scenario C (continuous grey arrows), the first step is the invasion of an unconditional TDMSC, either on the Y or an autosome (**2b**), that leads to the same intermediate state found in scenario A. The last step, is the same (**step 3**) than in scenario A. In scenario D (dashed grey arrows), the second (and final) step (**2b'**) is the invasion of a conditional TDMSC, here the mutation can be harboured by the Y chromosome or an autosome. Depending on the proximal mechanisms involved, the invasion dynamics could be different (e.g. on an autosome, the drive phenotype could be expressed in males, if for example they produce X and Y sperms more or less performant depending on female genotype, or in females, if the drive is due to a cryptic female choice).

Evolution of the X* in response to a selfish genetic element

According to this hypothesis, the **first step** (**1a**) is the invasion of a distorting Y chromosome, which will invade a population as long that it favours its own transmission (k>0.5, k is the Y chromosome transmission ratio) (Hamilton 1967). As it spreads, it will drive the population sex-ratio towards k, automatically selecting for a suppressor of drive (Burt and Trivers 2006), or any genetic element that will reduce the bias, here, the feminising X*.

Scenario A: We first considered the evolution of a feminising X* chromosome that has no effect on the transmission ratio of male sex chromosomes (**step 2a**). Such an X* will invade for any value of X*Y fitness $w > w_{crit1}(= \frac{1-k}{k}$, the threshold fitness, below 1 for k>0.5). Following the invasion of a driving Y, the X* could have invaded despite a low fitness of sex-reversed females, which is often observed in mammals. Interestingly, the stronger the bias (high k), the more tolerant the system will be to poor fitness of X^*Y females (low w), and the more female-biased the sex ratio becomes at equilibrium. In this scenario, the next step (**step 3**) is the invasion of a second distorter that affects the transmission ratio of male sex chromosomes only in crosses with X*Y females. The crucial question to ask concerning the evolution of this second driver, is which compartment of the genome could harbour a mutation that has this effect. It is likely that the bias toward transmission of the male X chromosome is due to a female driven effect (i.e. the drive phenotype is expressed when the mutation is harboured by a female). We therefore investigated three possibilities, and test the conditions allowing the invasion of the mutation on the X*, on the Y or on an

autosome. We found that the conditions for invasion are the same when considering a mutation on the Y or an autosome: such a mutant will invade as long as it reduces the proportion *k** of male Y chromosomes transmitted to the offspring of X*Y females ($k^* < k$). The consequence is a reduced production of X*Y females and YY embryos and an increase in production of males and XX* females. When considering the mutation on the X*, conditions for invasions are the same as for the Y or an autosome if X*Y females have a lower fitness than the XX and XX* ($w < 1$). However, if they have higher fitness ($w > 1$), the original X* will be replaced provided that the new one increases the strength of Y drive ($k^* > k$), in other words if it allows the production of more of the fitter X*Y females. It is interesting to see how the interests of different genomic compartments that are usually in conflict over the transmission of sex chromosomes and sex-ratio (e.g. autosomes and the Y chromosome) align here.

Scenario B: An X* affecting TDMSC in crosses involving X*Y females (**step 2a'**) will invade if $w > w_{crit2}$ (see figure and appendix). With *k>0.5*, w_{crit2} decreases for increasing values of *k* and decreasing values of *k**. This threshold value is inferior to 1 when *k>k** and in these conditions, always smaller than w_{crit1}, meaning that this path is even more tolerant to low fitness of X*Y females. The more the mutation on the X* reduces the transmission distortion of Y bearing gametes in crosses with X*Y females, the easier it will invade. With *k<0.5*, it is the opposite and w_{crit2} decreases with for increasing values of *k**.

Evolution of the X* ahead of the selfish genetic element(s)

This hypothesis relies on the fact that the X* could have evolved in the absence of a pre-existing Y drive. In agreement with theoretical work by Bull and Charnov (1977), we confirmed that the X* could have evolved if it provides a fitness advantage to X*Y females ($w > 1$) (**step 1b in figure 1**; see appendix).

Scenario C: In this scenario, we modelled the consecutive evolution of two TDMSC. The first one is unconditional, it affects the transmission ratio of male sex chromosomes alike in the three types of crosses (**step 2b**). We considered such a mutation could arise either on the Y chromosome or an autosome, and has an effect in males (the mutant phenotype (the drive) is expressed when the allele is harboured by a male). On an autosome, the mutation cannot invade in this context, as the only condition for invasion is *w<1*, which is

inconsistent with the conditions allowing maintenance of the X*. However the mutation could be held by the Y chromosome, assuming it favours its own transmission ($k>0.5$). This leads to the intermediate stage described in scenario A and **step 3**. If the mutation is harboured by the Y or an autosome, conditions for the invasion of a second segregation distorter are still: $k^* < k$ and as $w>1$, the replacement of the X* by one that has an effect on transmission distortion of male sex chromosomes will only be possible if $k^* > k$.

Scenario D: A mutation causing a conditional TDMSC could arise on the Y chromosome or on an autosome (**step 2b'**). Mechanistically, there are different ways in which a mutation could affect the transmission of male sex chromosomes in a conditional way. We considered one possibility for the Y, and another for the autosome. The mutation considered on the Y has an effect on transmission ratio of male sex chromosomes when in males (drive = k), and another effect when harboured by an X*Y female (drive = k^*), which overrides the male effect in crosses in which both the male and female bear the mutation[5]. The conditions for invasion are given in figure 1 and detailed in the appendix. Briefly, once again, high values of k and low values of k^* will facilitate the spread of the mutation : it is more likely to invade the more it favours the transmission of male Y chromosome in crosses with XX or XX* females and the X chromosome in crosses with X*Y females.

We assumed that the phenotype of the mutation considered for the autosome is dominant and is only expressed in females, meaning that the TDMSC is only present in the progenies of females bearing the mutation (regardless of male autosomal genotype), and its effect is dependent on sex chromosome complement (strength of distortion: k in XX and XX* females and k^* in the X*Y). We were unable to obtain simple analytical expressions in this case but numerical analyses suggest that such a mutation can invade if the transmission ratio of male Y chromosome is higher in crosses involving XX and XX* females ($k > k^*$), but invasion is not possible for high values of k^* or low values of k. The fecundity of X*Y females has little impact on these conditions (see appendix).

[5] Other possibilities will have to be considered, for example a "poison-antidote" system, in which the mutant phenotype in females (drive = k^*) is only expressed in crosses with males that themselves harbor the mutation.

DISCUSSION

Transmission distortion of male sex chromosomes and the unusual sex determination system in *Mus minutoides*

In the African pygmy mouse, the transmission ratio of male sex chromosomes is biased, due to a transmission distortion of male sex chromosomes, which depends on female genotype (conditional TDMSC, table 2). In particular, males that mate with the standard XX females transmit their Y chromosome to almost 80% of their offspring. This suggests that the feminising X* could have evolved in response to a selfish genetic element on the Y favouring its own transmission. Indeed, sex chromosome drivers have an effect on population sex-ratio, and can quickly lead the population to extinction if the strength of drive is strong (especially true for Y chromosome drivers, Hamilton, 1967). This situation provides the conditions for the spread of any type of modifier: in the case of a driving Y, a suppressor of drive (either on the X or an autosome), or an allele turning some genetic males into females, like the X* found in *Mus minutoides*.

In the light of these results and previous empirical results showing that X*Y females have a greater reproductive success (Saunders et al. 2014), we developed a set of theoretical models to explain the transition from a standard mammalian system to a polygenic SDS with conditional transmission distortion of male sex chromosomes. Results show that the feminising X* chromosome could very well have evolved in response to a driving Y chromosome (fig 1, blue arrows). In this scenario, a second driver favouring the transmission of male X chromosome when mated to an X*Y female could have evolved subsequently, either on the Y or an autosome (and on the X* if the fitness of X*Y females was lower than that of the other females), to reduce the reproductive cost linked to the production of non-viable YY embryos produced by these females. However, this is just substantial evidence and our models show that other scenarios are possible: the transmission modifier(s) could have evolved after the invasion of the X* sex determiner (figure 1, gray arrows). In that case, the trigger for the invasion of the mutation would have been a greater fitness of X*Y females (either thanks to pleiotropic effects of the mutation or linkage disequilibrium with female beneficial mutations). This type of scenario is however unlikely when considering that sex reversal is usually extremely detrimental in mammals: XY females tend to have poor fertility if not completely sterile (because of the loss of YY embryos and various and many other impairments like meiotic defects and further embryonic losses, Vernet et al. 2014 and references therein). With that in mind, our

model predicts that in the first scenario, the feminising mutation could have spread despite a reduced fitness of X*Y female (a relative fertility of 0.25 would have been enough for the invasion if males transmitted their Y chromosome to 80% of their offspring), which gives more credit to scenario 1. The greater performances of X*Y females have more likely evolved subsequently, by gradual accumulation of female beneficial genes and alleles (and loss of male beneficial ones) on the X*, as expected in the evolution of sex-limited chromosomes (Bachtrog 2006).

However, we cannot excluded that a completely different mechanism trigged the invasion of the feminising mutation, theoretical models show that interdemic selection and meta-population structure (Vuilleumier et al. 2007) or strong inbreeding (Maynard Smith and Stenseth 1978; Stenseth 1978) can favour the spread of a sex-reversal genes. But too little is known about the ecology and mating structures of the African pygmy mouse (Britton-Davidian et al. 2012) to affirm that these hypotheses are relevant. So even if the initial trigger for the evolution of the X* remains uncertain, overall, the models prove that both transmission distortion of male sex chromosomes and the greater fitness of X*Y females contribute to the maintenance of the system, by making the actual state more stable (increasing values of X*Y female fitness and the observed patterns of TDMSC, high *k* and small *k**, all drive the system away from the critical limit for maintenance).

To go further into the understanding of the evolution of the polygenic SDS of *Mus minutoides*, it would be interesting to study other populations. Sex-reversed females have been found from Southern up to Western African and molecular dating suggest the X* evolved almost 1 Mya (Veyrunes et al. 2013). The proportion of X*Y females seems to vary across localities (Veyrunes et al. 2013), suggesting that different transmission distorters might exist in different populations. Comparing the transmission ratio of male sex chromosomes and breeding success of X*Y females from localities populations will certainly help to identify the trigger of the evolution of the X*.

Selfish genetic elements and transitions between sex determination systems

There is growing evidence that selfish genetic elements are important drivers of evolutionary innovation (Hurst and Werren 2001; Taylor and Ingvarsson 2003; Werren 2011). They have for example recently been suggested to play a role in the evolution of polyandry (Price et al. 2008), androdioecy (Billiard et al. 2015), of course sex determination

(Werren and Beukeboom 1998), and it is likely that their role as a strong evolutionary force is greatly underestimated (Helleu et al. 2015).

Despite a strong theoretical framework (Kozielska et al. 2010), there are few examples unambiguously proving the role of sex-ratio distortion in sex determination modification. The most well described case is probably the role of the feminising endosymbiote *Wolbachia* in the turnover of sex determination systems in the woodlice *Armadillidium vulgare*. Different sex determination systems are found among different populations in the woodlice (several different female heterogametic systems, male heterogamety, and cytoplasmic sex determination), that have all evolved from an ancestral female heterogametic system as a response to the feminising effect of *Wolbachia* (reviewed in Cordaux et al. 2011). More speculatively, selfish genetic elements have been proposed to explain other transitions in Arthropods: the heterogametic transition in *Musca domestica* (Clark 1999) and the switch from an XX/X0 system to haplodiploidy or paternal genome elimination in some scale insects and sciarid flies (Haig 1993a,b).

Interestingly, all other suspected cases are found in mammals. Like in the African pygmy mouse, fertile X*Y females are found in the South American grass mouse *Akodon azarae* (Ortiz et al. 2009) and in the wood and collared lemmings *Myopus schisticolor* and *Dicrostonyx torquatus* (Fredga et al. 1977). The latter two have been shown to undergo biased transmission of sex chromosomes in some way. In the wood lemming, a mechanism of double non-disjunction of sex chromosomes in early oogonia of foetal ovaries in X*Y females results in a production of 100% X* bearing oocytes, and a small Y chromosome drive (0.54-0.59) exists in male collared lemmings (Gileva 1987). Once again, it is unclear whether sex chromosome drives in these species are the trigger for the modification of sex determination, but reveals that polygenic systems are favourable to the fixation of various types of sex chromosome drivers, whether they evolved before or after the novel sex determiner. There are other types of unusual sex determination systems in mammals. For two of them: *Talpa* moles (XY males and XX intersexes), and *Microtus oregoni* (XY males and X0 females), the modification of the mode of sex determination was also analysed theoretically in the light of genomic conflict (McVean and Hurst 1996; Charlesworth and Dempsey 2001), and theoretical results confirmed the potential implication of sex chromosome drivers in the evolution of both systems (driving X or Y for the first and driving X that causes the meiotic non-disjunction in both sexes for the second).

By adding *Mus minutoides* to the list of species in which genomic conflicts are involved in (and likely at the origin of) modification of sex determination, and showing that the two sex chromosome drives described make the X* more stable, we comfort the role of selfish genetic elements as an importance evolutionary force driving transitions in sex determination systems. A striking pattern found in mammals is that various types of transmission distortions of sex chromosomes are found in the different species with unusual SDS (conditional TDMSC in the African pygmy mouse, Y-drive in collared lemming, double non-disjunction of sex chromosomes in wood lemming and *Microtus oregoni*…), showing that these systems are probably more tolerant to sex chromosome drive than the standard male heterogametic system. For example, in a polygenic sSDS, a Y chromosome with 100% drive in a male would produce 100% sons in crosses with XX females, 50% sons in crosses with XX* females and 100% daughters in crosses with X*Y females, meaning that selective pressures for the evolution of suppressors are less strong.

Conditional transmission distortion of male sex chromosomes

The three types of crosses produced an excess of males, linked to biased transmission of male sex chromosomes. Quite surprisingly, we discovered that the direction and strength of the bias depends on female genotype (table 2). To our knowledge, it is the first time that such a conditional drive of sex chromosomes is described. The closest example we found was recently described by Billiard et al. (2015). It concerns the transmission pattern of male gametes in the androdioecious plant *Phillyrea angustifolia*. In this species there are males and two groups of hermaphrodites (H_a and H_b). Males are heterozygotes for the male determining allele M (M/m) and in crosses between males and hermaphrodites, there is a Mendelian transmission of the male alleles at this locus in crosses with H_a individuals, but a complete drive of the M allele in crosses with H_b individuals.

This kind of discovery of course brings along many questions, especially concerning the mechanisms underlying the transmission distortions. The only conclusion that can be drawn for the moment is that post-meiotic mechanisms must be involved as it is hard to conceive that males produce either more Y or X bearing sperms depending on the genotype of their partner. Here is an example of the mechanisms that could be involved if we assume that the X* evolved in response to a Y-chromosome drive: a selfish element on the Y chromosome could promote the transmission of Y chromosome by meiotic drive or by interfering with maturation of gametes harbouring the X chromosome, making them dysfunctional (the most widespread mechanism of transmission distortion, Taylor &

Ingvarrson 2003). The X-drive specific to crosses with X*Y females would have evolved subsequently, and could be due to female cryptic choice to reduce the production of lethal YY embryos. As differences exist between X and Y-bearing sperms in mammals (e.g. in surface proteins, Chen et al. 2012), X*Y females could favour fertilization by X-bearing sperms by rendering the genital environment hostile to Y-bearing sperm, or selectively abort "unwanted" embryos. The identification of the proximal mechanisms underlying the sex chromosome drives will likely lead to a better understanding the evolution of the unusual sex determination of *Mus minutoides*.

CONCLUSION

Assessing the evolutionary causes of a transitions among sex determination systems is hard. This is due to the fact the transitions are often rapid, and once the transition is over, the evidence is often gone. Fitness advantage of the emergent sex determiner cannot be evaluated as the ancestral genotypes have disappeared, and sex chromosomes drivers are either lost or not expressed in the new system. In this context, species with polygenic sex determination, like the African pygmy mouse, make valuable models to identify these causes. Here, using a combination of empirical and analytical approaches, we were able to show that sex chromosome drive is involved in the evolution of the feminising sex determiner found in this species, and very likely is the trigger of transition. The extent of the role of selfish genetic elements on transitions in sex determination system will benefit from the study of other species with polygenic sex determination, that are rare but can be found in mammals, fish, insects and plants (Moore and Roberts 2013).

Finally, this study has also revealed the existence of an unprecedented conditional distortion of male sex chromosomes, which clearly provides a great opportunity to learn more about the proximal and ultimate causes of the evolution of sex chromosome drive in mammals.

METHODS

Sex ratio at weaning and transmission ratio of sex chromosomes

The results presented here were obtained in our laboratory breeding facility, established in June 2010 from animals caught in Caledon Nature Reserve, South Africa (for full details see Saunders et al. 2014). Males and females were told apart by checking ano-genital distance and external genitalia at weaning, and sex chromosome complement of females was systematically assessed by PCR amplification of the Y-specific *Sry* gene following Veyrunes et al. (2010) and/or karyotyping (allowing discriminating X* and X).

Theoretical analyses

We analysed the invasion dynamics of mutations allowing to go from one state to the next (figure 1) using a sets of recurrence equations giving the frequencies of female genotypes at each generation, depending on their frequencies at the previous generation (see appendix for full details). We considered an infinite diploid population with random mating and non-overlapping generations. Such models are commonly used to investigate the evolutionary forces involved in transitions in sex determination systems (e.g. Bull and Bulmer 1981, Kosielska et al. 2010).

Sex is determined by one locus, with three recurrent alleles (X, X^* and Y) that make up one type of male (XY) and three types of females (XX, XX^* and X*Y).

Transmission of sex chromosomes is always random in females, and the ratio of Y chromosomes transmitted by males depends on female genotype. The strength of distortion (proportion of male Y chromosomes transmitted to the progeny) is denoted k in crosses with XX or XX* females, and k^* in crosses with X*Y females ($0 \leq k^{(*)} \leq 1$).

In agreement with empirical results (Saunders et al. 2014), XX females and XX* females have similar fitness, set to 1, and X*Y females have a relative potential fitness w.

REFERENCES

Bachtrog, D. 2006. A dynamic view of sex chromosome evolution. Curr. Opin. Genet. Dev. 16:578–585.

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T.-L. Ashman, M. W. Hahn, J. Kitano, I. Mayrose, R. Ming, N. Perrin, L. Ross, N. Valenzuela, and J. C. Vamosi. 2014. Sex Determination: Why So Many Ways of Doing It? PLoS Biol. 12:e1001899.

Beukeboom, L. W., and N. Perrin. 2014. The Evolution of Sex Determination. Oxford University Press.

Billiard, S., L. Husse, P. Lepercq, C. Godé, A. Bourceaux, J. Lepart, P. Vernet, and P. Saumitou-Laprade. 2015. Selfish male-determining element favors the transition from hermaphroditism to androdioecy. Evolution (N. Y). 683–93.

Britton-Davidian, J., T. J. Robinson, and F. Veyrunes. 2012. Systematics and evolution of the African pygmy mice, subgenus Nannomys: a review. Acta Oecol. 42:41–49.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park. CA.

Bull, J. J., and E. L. Charnov. 1977. Changes in the heterogametic mechanism of sex determination. Heredity (Edinb). 39:1–14.

Burt, A., and R. Trivers. 2006. Genes in conflict: the biology of selfish genetic elements. Cambridge.

Charlesworth, B., and N. D. Dempsey. 2001. A model of the evolution of the unusual sex chromosome system of *Microtus oregoni*. Heredity (Edinb). 86:387–394.

Chen, X., H. Zhu, C. Wu, W. Han, H. Hao, X. Zhao, W. Du, T. Qin, Y. Liu, and D. Wang. 2012. Identification of differentially expressed proteins between bull X and Y spermatozoa. J. Proteomics 1–9. Elsevier B.V.

Clark, M. E. 1999. The evolution of a neo-Y chromosome in the housefly, Musca domestica. University of Houston.

Cordaux, R., D. Bouchon, and P. Grève. 2011. The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends Genet. 27:332–41.

Fredga, K., A. Gropp, H. Winking, and F. Frank. 1977. A hypothesis explaining the exceptional sex ratio in the wood lemming. Hereditas 85:101–104.

Gamble, T., J. Coryell, T. Ezaz, J. Lynch, D. P. Scantlebury, and D. Zarkower. 2015. Restriction Site-Associated DNA Sequencing (RAD-seq) Reveals an Extraordinary Number of Transitions among Gecko Sex-Determining Systems. Mol. Biol. Evol. 32:1296–309.

Gileva, E. A. 1987. Meiotic drive in the sex chromosome system of the varying lemming , Dicrostonyx torquatus Pall. (Rodentia, Microtinae). Heredity (Edinb). 59:383–389.

Haig, D. 1993a. The evolution of unusual chromosomal systems in coccoids: extraordinary sex ratios revisited. J. Evol. Biol. 6:69–77.

Haig, D. 1993b. The evolution of unusual chromosomal systems in sciarid flies: intragenomic conflict and the sex ratio. J. Evol. Biol. 6:249–261.

Hamilton, W. D. 1967. Extraordinary Sex Ratios. Science (80-.). 156:477–488.

Hartigan, J., and P. Hartigan. 1985. The dip test of unimodality. Ann. Stat. 13:70–84.

Helleu, Q., P. R. Ge, and C. Montchamp-moreau. 2015. Sex Chromosome Drive.

Hurst, G. D., and J. H. Werren. 2001. The role of selfish genetic elements in eukaryotic evolution. Nat. Rev. Genet. 2:597–606.

Mahadevaiah, S. K., R. Lovell-Badge, and P. S. Burgoyne. 1993. *Tdy*-negative XY, XXY and XYY female mice: breeding data and synaptonemal complex analysis. J. Reprod. Fertil. 97:151–160.

Maynard Smith, J., and N. C. Stenseth. 1978. On The Evolutionary Stability Of The Female-Biased Sex-Ratio In The Wood Lemming (*Myopus schisticolor*): The Effect Of Inbreeding. Heredity (Edinb). 41:205–214.

McVean, G., and L. D. Hurst. 1996. Genetic conflicts and the paradox of sex determination: three paths to the evolution of female intersexuality in a mammal. J. Theor. Biol. 179:199–211.

Moore, E. C., and R. B. Roberts. 2013. Polygenic sex determination. Curr. Biol. 23:R510–2. Elsevier.

Ortiz, M. I., E. Pinna-Senn, G. Dalmasso, and J. A. Lisanti. 2009. Chromosomal aspects and inheritance of the XY female condition in *Akodon azarae* (Rodentia, Sigmodontinae). Mamm. Biol. 74:125–129.

Pennell, M. W., M. Kirkpatrick, S. P. Otto, J. C. Vamosi, C. L. Peichel, N. Valenzuela, and J. Kitano. 2015. Y fuse? Sex chromosome fusions in fishes and reptiles. PLoS Genet. 11:e1005237.

Price, T. a R., D. J. Hodgson, Z. Lewis, G. D. D. Hurst, and N. Wedell. 2008. Selfish genetic elements promote polyandry in a fly. Science 322:1241–1243.

Roberts, R., J. Ser, and T. Kocher. 2009. Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. Science (80-.). 326:998–1001.

Saunders, P. A., J. Perez, M. Rahmoun, O. Ronce, P.-A. Crochet, and F. Veyrunes. 2014. XY females do better than the XX in the african pygmy mouse, Mus minutoides. Evolution (N. Y). 68:2119–27.

Stenseth, N. C. 1978. Is the female biased sex ratio in wood lemming Myopus schisticolor maintained by cyclic inbreeding? Oikos.

Taylor, D. R., and P. K. Ingvarsson. 2003. Common features of segregation distortion in plants and animals. Genetica 117:27–35.

Van Doorn, G. S. 2014. Evolutionary transitions between sex-determining mechanisms: A review of theory. Sex. Dev. 8:7–19.

Van Doorn, G. S. 2014. Patterns and mechanisms of evolutionary transitions between genetic sex-determining systems. Cold Spring Harb. Perspect. Biol. 6.

Vernet, N., M. Szot, S. K. Mahadevaiah, P. J. I. Ellis, F. Decarpentrie, O. a Ojarikre, Á. Rattigan, T. Taketo, and P. S. Burgoyne. 2014. The expression of Y-linked Zfy2 in XY mouse oocytes leads to frequent meiosis 2 defects, a high incidence of subsequent early cleavage stage arrest and infertility. Development 141:855–66.

Veyrunes, F., P. Chevret, J. Catalan, R. Castiglia, J. Watson, G. Dobigny, T. J. Robinson, and J. Britton-Davidian. 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. B 277:1049–56.

Veyrunes, F., J. Perez, S. N. C. Paintsil, E. Fichet-Calvet, and J. Britton-Davidian. 2013. Insights into the evolutionary history of the X-Linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked Sry gene. Sex. Dev. 7:244–52.

Vicoso, B., and D. Bachtrog. 2015. Numerous transitions of sex chromosomes in Diptera. PLoS Biol. 13:e1002078.

Vuilleumier, S., R. Lande, J. J. M. Van Alphen, and O. Seehausen. 2007. Invasion and fixation of sex-reversal genes. J. Evol. Biol. 20:913–920.

Werren, J. H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U. S. A. 108:10863–10870.

Werren, J. H., and L. W. Beukeboom. 1998. Sex Determination, Sex Ratios, and Genetic Conflict. Annu. Rev. Ecol. Sys. 29:233–261.

Manuscrit 3: “Conditional transmission distortion of male sex chromosomes in the African pygmy mouse: the search for proximal mechanisms”

Afin de mieux comprendre la distorsion de transmission conditionnelle des chromosomes sexuels mâles chez *Mus minutoides* (Manuscrit 2), nous avons démarré une étude visant à identifier les causes proximales sous-tendant les biais observés. Etant donné que les sex-ratios sont mesurés lors du sevrage, divers mécanismes pourraient être responsables des de ces biais, allant d'une distorsion méiotique à une mortalité différentielle des juvéniles portant le X ou le Y. Le fait que la distorsion de transmission soit dépendante du génotype femelle suggère même que plusieurs mécanismes physiologiques pourraient être impliqués. L'objectif de cette étude est d'identifier le(s) compartiment(s) biologique(s) au sein duquel(s) les biais se produisent : (i) dans le compartiment mâle, dans lequel les biais pourraient être causés par une distorsion méiotique menant à une production non équilibrée de spermatozoïdes X et Y, ou une mortalité différentielle des spermatozoïdes dans le tractus mâle, (ii) dans le compartiment femelle pré-fécondation, à cause de différences de performances entre les spermatozoïdes X et Y ou d'un choix cryptique de la femelle lors du transport des spermatozoïdes ou lors de la fécondation, et (iii) dans le compartiment post-fécondation, lié à une mortalité embryonnaire ou juvénile différentielle.

Plusieurs expériences et observations ont été réalisées afin de déterminer quel(s) compartiment(s) est (sont) impliqué(s) dans les distorsions, les résultats sont résumés dans la table ci-dessous.

	Etude de la contribution de…	Mesure	Résultats
Spermatogénèse	Distorsion méiotique	Ratio de spermatocytes X et Y lors de la méiose II	Autant de spermatocytes X et Y: pas de distorsion méiotique
	Mortalité différentielle des spermatozoïdes lors de la maturation	Ratio de spermatozoïdes X et Y matures	Autant de spermatozoïdes X et Y transmis à la femelle
		…	
	Mortalité précoce des embryons	Mesure du taux de mortalité embryonnaire à 10-15 jours, comparaison avec le ratio de transmission des chromosomes sexuels males	Pas de correlation entre taux de mortalité embryonnaire et ratio de transmission: pas de mortalité préferentielle d'un type d'embryon.
Sevrage	Mortalité embryonnaire tardive et juvénile	Comparaison du sex-ratio embryonnaire à 10-15 jours au sex-ratio observe au sevrage	Sex-ratio embryonnaire = sex-ratio au sevrage

Récapitulatif des résultats concernant la recherche des causes proximales des distorsions de transmission.

Ces résultats suggèrent que les distorsions de transmission des chromosomes sexuels mâles n'ont ni lieu dans le compartiment mâle, ni dans le compartiment post-fécondation, ce qui signifie que les mécanismes agissent dans les voies génitales de la femelle. Selon le génotype de la femelle, les spermatozoïdes X et Y pourraient avoir une survie différentielle lors de l'acheminement jusqu'au site de fécondation, ou une probabilité différente d'être impliqués dans la syngamie.

Ces résultats sont un premier pas pour comprendre les mécanismes impliqués dans les distorsions de transmission des chromosomes sexuels mâles observées chez *Mus minutoides*. Des tentatives visant à aller plus loin dans l'identification des causes proximales ont été entreprises (fécondations in vitro réalisées par Manuel Avilés de l'université de Murcia en Espagne, inséminations artificielles), sans succès, et une expérience visant à comparer la survie des spermatozoïdes portant le chromosome X ou le Y dans les tractus génitaux des différentes femelles va bientôt démarrer.

Species	Distorter	Mechanism of drive
House mouse	Autosomal	Poor motility, swimming impairments (Olds-Clarke and Johnson 1993)
Mus musculus	"t-haplotype"	Delay in penetrating the *zona pellucida* (Johnson et al. 1995)
Drosophila[1]	Autosomal "SD"	Degeneration of spermatids (Kettaneh and Hartl 1980)
Drosophila[1]	X-drive "SR"	*D. simulans:* Y chromatids fail to separate at meiosis II (Cazemajor et al. 2000)
Stalk-eyed flies	X-drive	No elongation of most Y-bearing sperms (Wilkinson and Sanchez 2001)
Telopsis dalmanni		
Mosquitos	Y-drive ("D")	X chromosome breaks during the first meiotic division
Aedes aegypti & Culex pipiens		(Newton et al. 1976; Sweeny and Barr 1978)
House mouse	X-drive[2]	Spermhead abnormalities, mobility loss and reduced ability to fertilize (Ward
Mus musculus	Slx/Sly conflict	and Burgoyne 2006)

Table 1. Overview of some of the segregation distorters found in animals

[1]Several species are affected.

[2]Strength of drive actually depends on the ratio of multicopy genes *Slx vs*. *Sly*, and Y-drive is also possible (Cocquet et al. 2012).

Conditional transmission distortion of male sex chromosomes in the African pygmy mouse: the search for proximal mechanisms

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INTRODUCTION

Thanks to Mendelian segregation of chromosomes at meiosis, the two alleles at a locus are transmitted to the progeny at a 1:1 ratio. At heterozygous loci, it happens that one of the alleles is transmitted more often than the other. This phenomenon is called a transmission distortion, and is often caused by a selfish genetic element that promotes its own transmission at the expense of the other allele. Transmission distortion can affect loci on autosomes as well as on sex chromosomes. They are easier to detect on sex chromosomes, as they have an effect on sex ratio. They also have more profound evolutionary consequences, especially in species in which sex chromosomes have stopped recombining, as the driving allele will repeatedly drag the whole chromosome with it during the drive, increasing its frequency, and potentially leading the population to extinction (Hamilton 1967). Because of the strong genomic conflicts they generate, sex chromosome transmission distorters have been recognized as an important evolutionary force (Werren 2011): they have been proposed to be involved in the evolution of polyandry and androdioecy and to drive transitions among sex determinations systems (Werren and Beukeboom 1998; Price et al. 2008; Billiard et al. 2015). However, theyare rare. Their scarcity if thought to stem from the fact that their higher transmission rate and the deleterious effects associated (e.g. gamete killing) generate a selective pressure at other loci to suppress their action, and many known transmission distorters segregate in natural populations along with suppressors of drive (Burt and Trivers 2006). In animals, only a handful of transmission distorters have been described, and they seem to have many common features (reviewed in Taylor and Ingvarsson 2003): males are more affected than females, and most of the time, the drive is due to the disruption of spermatogenesis in gametes that don't carry the driver. Table 1 shows an overview of several well described segregation distorters in animals and the proximal mechanisms involved in the drive.

Recently, we identified a surprising novel case of transmission distortion in a mammal, the African pygmy mouse *Mus minutoides* (Chapter 1, part 2), a close relative of the house mouse and one of the rare mammals with an unusual sex determination system (Veyrunes et al. 2010). While all males have a typical XY sex chromosome complement, there are three types of females: XX, XX* and the sex-reversed X*Y (The X* harbours a still unknown sex determiner that overrides the masculinising effect of the Y chromosome in X*Y embryos). The three types of females produce more males than expected, due to a transmission distortion of male sex chromosomes. What makes this system so unique and interesting, and a great model to better understand the proximal mechanisms involved in sex chromosome drive in mammals, is that this sex chromosome drive depends on the genotype of the female with which the male mates. In crosses with XX or XX* females, males transmit their Y chromosomes more often (roughly 80% of the time), while in crosses with X*Y females, it is the male X chromosome which is favoured (64%). There are potentially two different mechanisms that act to produce this output, and as sex-ratio was measured at weaning, a profusion of mechanisms, ranging from meiotic drive to differential mortality of pups bearing paternal X or Y chromosomes, could be responsible for the drives.

To start deciphering the mechanisms underlying this remarkable segregation distortion, we defined three major biological compartment in which the bias could occur: (i) male compartment (which spans from meiosis to ejaculation), (ii) female pre-fertilization compartment (from the entry of sperm cells in female reproductive tracts to the fusion of gametes) and (iii) post-fertilization compartment (from caryogamy to weaning), and conducted a set of analyses to determine in which compartment(s) the ratio of males sex chromosomes goes from 50:50 (Y:X) to 80:20 or 36:64, depending on female genotype.

We analysed:

(i) The ratio of X and Y spermatocytes to test whether biases could be linked to meiotic drive.

(ii) The ratio of X and Y mature sperm to assess the proportion of X and Y sperm transmitted to females at mating.

(iii) Early embryonic survival rates, in relation with paternal chromosome harboured by surviving embryos, in order to determine if the shift is caused by early differential mortality of X and Y bearing embryos.

(iv) Mid-term embryo sex-ratio, and compared it to sex-ratio at weaning to find out whether sex-ratio biases are linked to differential mortality at a late embryic stage or in pups.

This first step is necessary to unravel the proximal mechanisms responsible for the conditional sex chromosome drive of male African pygmy mice, and more generally, might extend our knowledge about transmission distortion in mammals.

METHODS

All analyses presented here are based on results obtained in our laboratory breeding stock (described in Saunders et al. 2014). Individual sacrificed for the purpose of the experiments were killed by CO2 inhalation. All experiments were performed in accordance with European guidelines and with the approval of the Ethical Committee on animal care and use of France (No. CEEALR- 12170).

X and Y ratio of metaphase II spermatocytes

Air dried preparations of testes (for 6 males) were made based on work by Evans et al. (1964). Both testes were placed in 1% sodium citrate in a watch glass at room temperature, and tunicas were removed. Seminiferous tubules were shredded with curved forceps and transferred to a 15ml centrifuge tube completed to 8ml with sodium citrate. The tube was incubated at 37°C for 20 minutes and centrifuged three times (10 minutes at 1200 rpm then 5 minutes at 2100 rpm twice), supernatant being discarded and replaced with 6ml of sodium citrate after the first two centrifugations. After the last centrifugation, supernatant was replaced by 2ml fixative solution (1:3 acetic acid:methanol) and stored at -20°C until spreading (15µl on a dry glass slide). Slides were stained with Giemsa prior to scoring using an optical microscope. For each individual, 50 metaphase II spermatocytes were scored: the sex chromosome present in each nuclei was assessed, abnormalities were reported, and departure for 50:50 assessed using binomial tests.

X and Y ratio of mature sperm

Ratio of X *vs*. Y bearing spermatozoa was assessed in the *cauda epididymides*, where they are stored before ejaculation (see Jones, 1999 and references therein), using Fluorescent *in-situ* hybridization (FISH) with X and Y specific probes simultaneously. This

protocol is based on the protocol used in Lowe et al. (1996). The six males used for this experiment had fathered at least one litter, with one of the three types of females.

Slide preparation: Sperm were collected from both *cauda epididymides* were immediately spread on a dry glass slide. Slides were stored at room temperature (RT) for up to four weeks before hybridization. Just before hybridization, decondensation of sperm heads was performed: slides were incubated at RT in freshly prepared 10mM dithioerythritol (DTT) and 4mM LIS (both in 0.2mM Tris-Hcl ph8) for 5 and 60 min respectively (slides were air dried between the two washes). Slides were then quickly immersed in distilled water and air-dried again. Prior to hybridization, slides were denatured by incubation in 70% formamide, 2SSC pH7 at 75°C for 5 minutes, and dehydrated through a cold ethanol concentration series (70, 80,100%), 2 minutes each.

Probes: Both X and Y probes were mouse Bacterial Artificial Chromosomes (BAC), obtained from the Children's Hospital Oakland Research Institute (CHORI, CA, USA) from the Chori-29 library (X: CH29 168-N4, Y: CH29 604-J10). Probes were labelled by nick-translation (Roche Nick-mix), incorporating digoxigenin-11-dUTP and biotin-14-dUTP in DNA for chromosome X and Y probes respectively. The specificity of probes was assessed on metaphases obtained from bone marrow of yeast-stimulated animals (Lee and Elder 1980) (Figure S1). Probes were denatured (in parallel to slide denaturation) at 70°C for 10 min and preannealed at 37°C for 30 min.

Hybridization: 10µl of each preannealed probe mix was applied on to the sperm smears and slides were incubated overnight. Post-hybridization washes were carried out at 37-39°C in 2SSC for 3 min and in 4XT (4xSSC, 0.06µM Tween 20) for 5 min. Probes were detected by αbiotin and αdigoxigenin antibodies carrying respectively CY3 and FITC fluorochromes. After a 30 minutes incubation at 37°C, nuclei were counterstained with Vectashield mounting medium with 1.5µg/ml DAPI (Vector Laboratories) and slides were cover by a glass coverslip.

The slides were analysed under a Zeiss Axioplan 2 microscope and at least 200 sperm cells were manually scored on each slide, and departure from the expected 50-50 X-Y ratio was assessed using binomial tests.

Early embryonic mortality

Ovaries and mid-term embryos (between 10-15 days post coitum (DPC)) of 50 pregnant females (25 XX* and 25 X*Y) were collected (no XX females were used since they are produced in low frequency and rare in our breeding colony, but we assume that results would be similar to those obtained in XX* females). For each female, embryonic survival rate was assessed by comparing the number of embryos found in the oviducts and the number of *corpora lutea* (proxy of the number of oocytes produced) seen on the surface of both ovaries under a binocular microscope. Embryos were genotyped using *Sry* PCR or karyotyped and the ratio of mid-term embryos carrying their father's Y chromosome was assessed in each embryonic litter.

If the transmission distortions were due to a differential survival of X and Y bearing embryos, we would expect to find a correlation between embryonic survival and transmission ratio of the father's chromosome: transmission ratio should be close to 50:50 if no (or few) embryos are lost, and the strongest bias would be find in females with the lowest embryonic survival. Correlation between embryonic survival rate and proportion of surviving embryos bearing a Y chromosome was tested with Spearman's rank correlation test.

Late embryonic or pre-weaning mortality

The sex-ratio of embryos of the 50 females sampled previously plus 7 and 5 other XX* and X*Y pregnant females was assessed to find out whether sex-ratio could be shifted during late embryonic life and/or during early postnatal life, the sex-ratio of these "mid-term" embryos genotyped (see above) was evaluated for XX* and X*Y mothers, and compared to the estimated sex-ratios at weaning, using binomial tests.

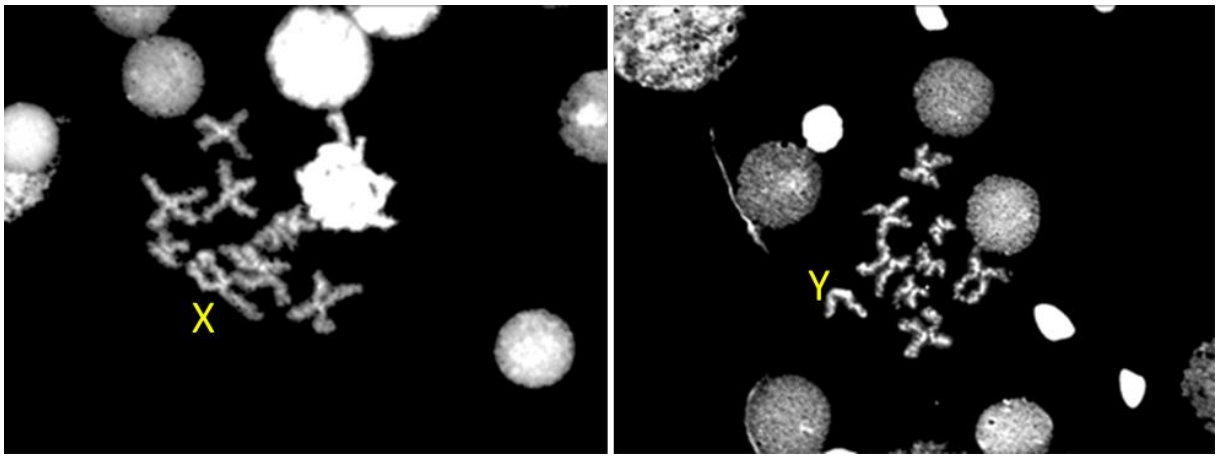


Figure 1. X and Y bearing spermatocytes at metaphases II (x1000)**.**

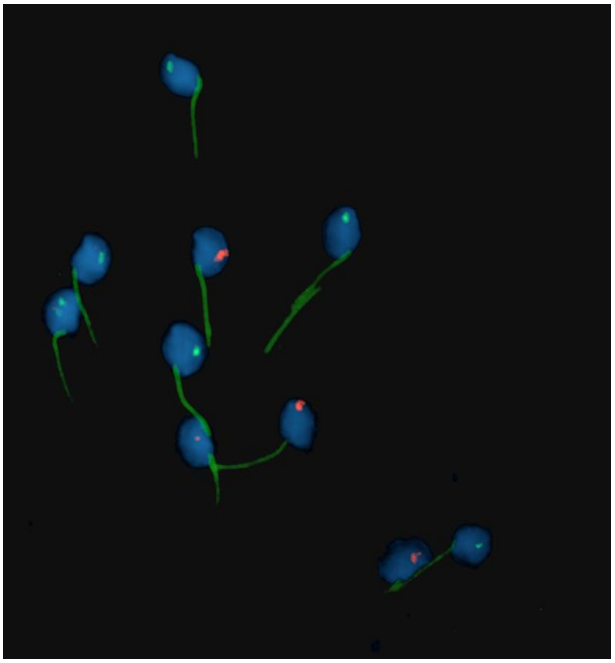


Figure 2. Mature sperm cells. Y chromosomes are stained with CY3 (red), X chromosomes are stained with FITC (green). Nuclei are counterstained in DAPI (blue) (x630)**.**

RESULTS

X and Y ratio metaphase II spermatocytes

Counts of X and Y bearing spermatocytes at metaphase II (figure 1) revealed no departure from the expected 50:50 ratio (mean Y-ratio: 0.507 +/- 0.03 (mean +/- sd), table S1). On some slides, some metaphases could not be scored, most of the time because of poor overall quality of the slide preparation. Also, there were no signs of degeneration or breakage of the sex chromosomes (as seen in the Y-drive transmission distortion in mosquitos, Newton et al. 1976, Sweeny & Barr 1978).

X and Y ratio of mature sperm

FISH efficiency, evaluated as the ratio of stained sperm heads (non ambiguously) to the total number of heads counted was high (96.83+/-2.46%) (Table S2). The average number of cells counted was 211, and average Y chromosome ratio was of 0.503+/-0.017. None of the males tested departed from a balanced ratio (Table S2), suggesting that males transmit a 50:50 ratio of X and Y bearing sperms to females, and that regardless of female genotype (figure 2).

Early embryonic mortality

The average *corpora lutea* count (proxy for the ovulation rate) was of 5.36 +/- 2.07 in XX* females and 7.72 +/- 2.24 in X*Y females and the average embryo number found in female tracts was of 3.72 +/- 0.98 in the XX* and 4.80 +/- 1.55 in the X*Y (implanted but aborted embryos were not counted). These estimates were used to assess an average mid-term embryonic survival rate: 0.75 +/- 0.22 in XX* and 0.65 +/- 0.18 in X*Y.

We found no correlation between embryonic survival rate and male Y transmission ratio for the embryos sampled in XX* nor X*Y females (rho=0.17, p=0.40 and rho=-0.21, p=0.30) (Figure 3) which shows that the male sex chromosome distortion is not due to a higher mortality of embryos bearing their father's X in the progeny of XX* females and their father's Y in the progeny of X*Y females. If it did, we would have observed a negative correlation for XX* female data, and a positive one for X*Y female data.

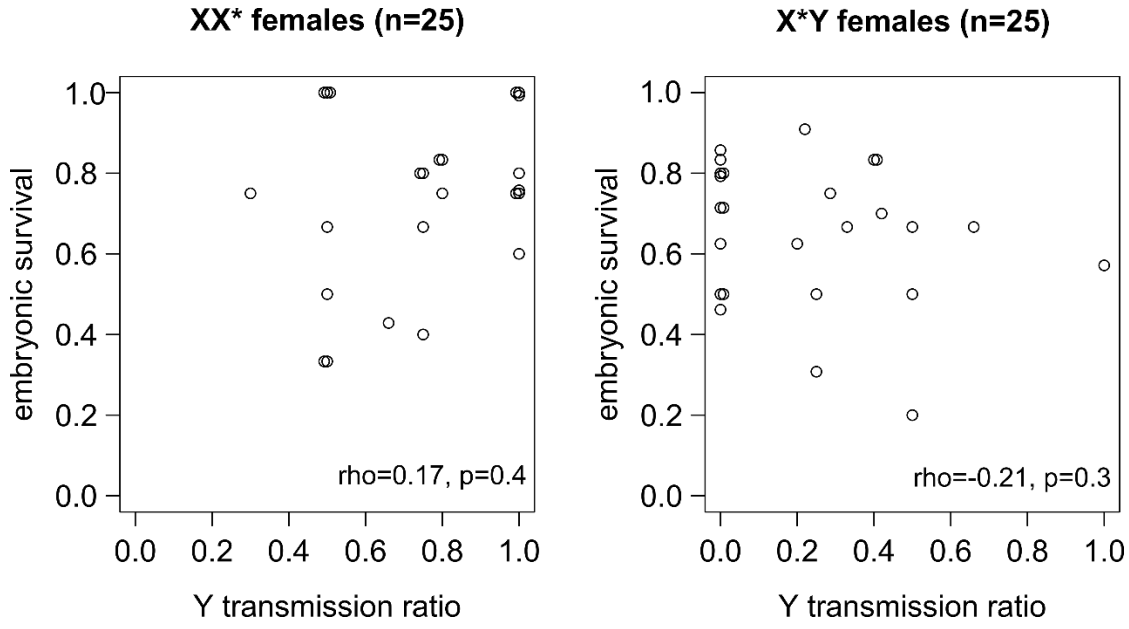


Figure 3. Embryonic survival *vs.* Y transmission ratio statistic: Spearman's rank correlation test.

Late embryonic or pre-weaning mortality

Most embryos collected for the embryonic survival analysis were sexed (81.6 and 66.5% percent of embryos found in XX* and X*Y females respectively, see Table S3) and sex-ratio amongst these embryos was assessed: in XX* mothers, 52 out of 138 sexed embryos were males (37.7%), and in X*Y mothers 59 out of 113 sexed embryos were males (40.4%). These findings were compared to the observed sex-ratios at weaning respectively (37.2 and 42.2%, see part 2 of chapter 1): binomial tests failed to identify differences between embryo sex-ratio and weaning sex ratio (p-value: 0.93 (IC=0.296-0.463) for XX* mothers, p-value: 0.68 (IC= 0.323-0.488) for X*Y mothers) suggesting that mortality that occurs in late embryonic life and early post-natal life of these mice is not "sex chromosome complement biased" and therefore does not influence the biased transmission of sex chromosomes in males.

DISCUSSION

Overview of the potential mechanisms for drive of male sex chromosomes

All known segregation distortions of male sex chromosomes in animals are caused by paternal X-Y intragenomic conflicts: the sex chromosome driver favours its own transmission by killing or disrupting the proper development of non-bearer gametes during male meiosis or spermiogenesis. However, a biased transmission of male sex chromosomes could also be caused by maternally driven factors. In that case, it is a genetic element in the female genome that would favour or disfavour the transmission of paternal X or Y chromosome. Here I review all the potential mechanisms that could be involved in a biased transmission of male sex chromosomes in mammals, spanning from male meiosis to weaning. Some mechanisms have already been described, and others are just speculative.

Male reproductive tracts. A transmission distorter of sex chromosomes can act during male **meiosis**, as seen in several Mosquitos species with Y-drive in which degradation or breakage of X chromosomes can be observed at meiosis I (Newton et al. 1976; Sweeny and Barr 1978). In "Sex-ratio" *Drosophila simulans*, a non-disjunction of Y chromatids at anaphase II, resulting in the failure of affected spermatids to develop, is responsible for X-drive (Cazemajor et al. 2000). **Post-meiotic** transmission distortion often involves selective elimination sperm cells in seminiferous tubules or during their maturation in the *epididymis*. This can result in apoptosis of maturing sperm cells (mechanism suggested to explain X-drive in stalk-eyed flies, Reinhardt et al. 2014). If drive is complete, males would transmit only gametes bearing the distorter to females at mating.

Female reproductive tracts pre-fertilization. The effects of a paternal X-Y intragenomic conflict can span beyond male reproductive tracts, if the development of sperm cells not carrying the driver is not stopped but altered, leading to a functional disruption. Many traits of spermatozoa can be affected: motility (like in the t-haplotype, Olds-Clarke 1996), acrosome integrity or swimming velocity. In that case, a 1:1 ratio of X/Y-bearing sperms will be found in the *epididymis* or an ejaculate.

Concerning maternally driven mechanisms, a biased transmission could be due to a bias in sperm use by females. Such "female cryptic choice" represents an important potential mechanism of post-copulatory sexual selection in polyandrous species (Eberhard 2009). However, the ability of females to select sperm within a single ejaculate remains

largely unexplored (but has been suspected in drosophila, Denell and Judd 1969; Mange 1970; Long and Pischedda 2005), and requires that females (i) are able to discriminate sperm cells based on the sex chromosome they harbour and (ii) dispose of mechanisms to favour transmission of X or Y bearing gametes.

(i) For females to be able to favour sperm based on the sex chromosome they harbour, a correlation between their sex chromosome complement and phenotype must exist, and it does. In bulls, X and Y sperms bear different phenotypic markers (Chen et al. 2013): 14 proteins have been found to be differentially expressed between the two types of gametes: some of them involved in functions such as cell defence and resistance to stress and production of surface proteins, cues that could be used by the females to favour one or the other type of gametes. Also, it was recently shown that oviductal gene expression is affected by X and Y-bearing spermatozoa, suggesting the existence of a sex-specific-sperm recognition system (Almiñana et al. 2014).

(ii) The potential mechanisms that could be used by female mammals to select sperm cells are numerous. It was recognized in the last decade that female reproductive tracts in mammals play an active role in sperm transport, storage and fertilization (reviews in Suarez 2008; Holt and Fazeli 2010; Ikawa and Inoue 2010; Coy et al. 2012), and act "as a highly effective semen analysis laboratory that is capable of distinguishing "good" and "excellent" sperm quality at the level of the individual spermatozoon" (Holt & Fazeli, 2010). For example, in the oviduct, there are several filters that favour the fittest sperms: the uterotubal junction has a transfer rate of 1 out of 10,000 sperm cells, and "selects" sperm based on their motility but also based on surface proteins. In the Isthmus, that plays the role of reservoir before fertilization, sperm are bound to the surface of the epithelium. Several molecules (both at the surface of sperm cells and epithelium cells) are involved in this interaction, which is essential to preserve quality of sperm cells, and to hyperactivate them to give them a chance to fertilise an egg.

So females could act on the transmission ratio of male sex chromosomes by preventing X or Y bearing sperms of passing one of the barriers separating them from the fertilization site, or by affecting their fertilization ability.

Interaction between male and female gametes. As an extension of mechanisms described in the previous paragraphs, both paternal and maternal factors could affect interactions between sperm cells and eggs and bias the transmission ratio of X and Y

bearing sperms. Before the sperm cell comes into contact with the oocyte, it has to go through a matrix surrounding the female gamete: the *cumulus oophorus*, which requires the presence of certain molecules at the surface of sperm cells. Then comes the acrosomal reaction, that only capacitated sperms can achieve, the contact with the *zona pellucida*, that involves a variety of proteins, the fusion of membranes, caryogamy… So many processes that could be affected by differences between X and Y sperms and/or cryptic female choice.

Post-fertilization. Transmission distortion could be due to differential mortality/implantation/resorption rates of X and Y bearing embryos and even differential survival rates of offspring with different genotypes after birth. Indeed, it has been shown that the effect of selfish genetic elements can span to after fertilization. In some beetles, an autosomal maternal-effect killer (*Medea*) gains a transmission advantage by killing larvae that do not bear that element (Beeman et al. 1992). Similarly, a locus on *Mus musculus* chromosome 1 (*HSR*) also is a maternal-effect killer (Weichenhan et al. 1996), and the embryos not carrying the factor are more often resorbed during embryogenesis. Another type of maternal effect proposed to affect transmission ratios is "gestational drive" (Haigt 1996) , caused by alleles that induce females to invest more in offspring in which they are present. More surprisingly, differential mortality rates between X and Y bearing embryos can also result from the impact of paternal intragenomic conflicts: so called zygotic drive, functionally equivalent to drive acting during meiosis or spermiogenesis, except that it operates after fertilization (Rice et al. 2008; Rice 2014), could also affect the transmission ratio of paternal sex chromosomes.

Transmission distortion takes place in the female tracts, pre-fertilization.

Female African pygmy mice produce more sons than expected because of an unprecedented transmission distortion of male sex chromosomes. Males mated to either XX or XX* females transmit their Y more often and those mated to X*Y females their X more often. An abundant number of mechanisms could be responsible for these sex chromosome drives, ranging from male meiosis to weaning.

By studying the ratio of paternal X and Y chromosomes at several key steps along this sequence, we were able to show that the switch from a balanced to unbalanced X:Y transmission ratio takes places in female reproductive tracts, sometime between mating and fertilization. Male compartment was excluded as the ratio of gametes found in *cauda*

epididymis was 1:1, providing the evidence that males transmit as many X and Y bearing sperms in their ejaculate, regardless of female genotype. Also, there was no correlation between embryonic survival and transmission ratio, and sex ratio of mid-term embryos was identical to sex-ratio at weaning, excluding the involvement of the post-fertilization compartment. These results narrow down the search for the mechanisms responsible for the biased transmission of male sex chromosomes. Two types of mechanisms remain: (i) a differential transport of X and Y bearing spermatozoa to the site of fertilization and (ii) differences in fertilization ability.

The conditional nature of sex chromosome drive in *Mus minutoides* suggests that several mechanisms could be operating, but our current results are not sufficient to reject the hypothesis that a single mechanism is involved. Here a couple different ways in which the drives could be accomplished. The first scenario involves both male and female effects, and the second and third ones rely on a unique mechanism: a male effect and a female effect respectively. (i) It was proposed that the feminizing X* chromosome evolved in response to a Y chromosome drive in males to restore a balanced sex-ratio, and that X*Y females subsequently evolved the capacity of favouring X bearing sperms to reduce to production of non-viable YY embryos (see manuscript 2). Conforming to this hypothesis, the drive of male Y chromosome when mated to XX and XX* females could be cause by a selfish genetic element giving a transmission advantage to spermatozoa bearing it by interfering with the development of X-bearing gametes, making them less likely to fertilize eggs (relying on mechanisms similar to those found in the mouse t-haplotype for example). A female driven effect in X*Y females could have evolve subsequently, inversing this transmission ratio by selectively killing Y bearing sperms or by favouring the transport of gametes that harbour the X chromosome. (ii) Alternatively, different male effect factors could provide X and Y bearing sperms with greater chances of fertilizing the eggs depending on maternal phenotype, in that case, there is no need for an "active" effect of the female (it does however require that pre-fertilization genital environment differs along with female genotype). (iii) Finally, transmission distortion could be entirely female driven: if female driven effects favour Y bearing sperm in a XX or XX* context, and X bearing sperm in X*Y females (in this scenario, there is no drive through males, but phenotypic differences between X and Y bearing sperm cells are required).

Now that transmission distortions have been found to take place in the female compartment pre-fertilization, to pinpoint the exact mechanism(s) operating, more

elaborate analyses are required. Sorting sperm based on their sex chromosome complement using flow cytometry (Garner 2006) could allow to compare their morphology and detect any abnormalities (However, informal observations of gametes freshly collected from cauda epididymis were made, and extremely few cells seemed to have morphology defects or were completely immotile). Performances (i.e. acrosome integrity, motility and swimming velocity) could be tested in a neutral buffer medium, to evaluate the existence of male effects, and a suspension containing isolated female tissues from either XX/XX* females or X*Y females, to test for female effects. Finally, to test for differential mortality, sperm-FISH could be combined to TUNEL assay (in-situ cell death detection) in the two types of media.

REFERENCES

Almiñana, C., I. Caballero, P. Heath, S. Maleki-Dizaji, I. Parrilla, C. Cuello, M. Gil, J. Vazquez, J. Vazquez, J. Roca, E. Martinez, W. Holt, and A. Fazeli. 2014. The battle of the sexes starts in the oviduct: modulation of oviductal transcriptome by X and Y-bearing spermatozoa. BMC Genomics 15:293.

Beeman, R. W., K. S. Friesen, and R. E. Denell. 1992. Maternal-effect selfish genes in flour beetles. Science 256:89–92.

Billiard, S., L. Husse, P. Lepercq, C. Godé, A. Bourceaux, J. Lepart, P. Vernet, and P. Saumitou-Laprade. 2015. Selfish male-determining element favors the transition from hermaphroditism to androdioecy. Evolution (N. Y). 683–93.

Burt, A., and R. Trivers. 2006. Genes in conflict: the biology of selfish genetic elements. Cambridge.

Cazemajor, M., D. Joly, and C. Montchamp-Moreau. 2000. Sex-ratio meiotic drive in Drosophila simulans is related to equational nondisjunction of the Y chromosome. Genetics 154:229–236.

Chen, X., R. McClusky, Y. Itoh, K. Reue, and A. P. Arnold. 2013. X and Y chromosome complement influence adiposity and metabolism in mice. Endocrinology 154:1092–104.

Cocquet, J., P. J. I. Ellis, S. K. Mahadevaiah, N. a Affara, D. Vaiman, and P. S. Burgoyne. 2012. A genetic basis for a postmeiotic x versus y chromosome intragenomic conflict in the mouse. PLoS Genet. 8:e1002900.

Coy, P., F. a García-Vázquez, P. E. Visconti, and M. Avilés. 2012. Roles of the oviduct in mammalian fertilization. Reproduction 144:649–60.

Denell, R. E., and B. H. Judd. 1969. Segregation distorter in D. melanogaster males: An effect of female genotype on recovery. MGG Mol. Gen. Genet. 105:262–274.

Eberhard, W. G. 2009. Postcopulatory sexual selection: Darwin's omission and its consequences. Proc. Natl. Acad. Sci. U. S. A. 106 Suppl :10025–10032.

Evans, E. P., G. Breckon, and C. E. Ford. 1964. An Air-Drying Method for Meiotic Preparations from Mammalian Tests. Cytogenetics 3:289–94.

Garner, D. L. 2006. Flow cytometric sexing of mammalian sperm. Theriogenology 65:943–57.

Haigt, D. 1996. Gestational drive and the green-bearded placenta. 93:6547–6551.

Hamilton, W. D. 1967. Extraordinary Sex Ratios. Science (80-.). 156:477–488.

Holt, W. V, and A. Fazeli. 2010. The oviduct as a complex mediator of mammalian sperm function and selection. Mol. Reprod. Dev. 77:934–43.

Ikawa, M., and N. Inoue. 2010. Fertilization: a sperm's journey to and interaction with the oocyte. J. Clin. Invest. 120.

Johnson, L. R., S. H. Pilder, J. L. Bailey, and P. Olds-Clarke. 1995. Sperm from Mice Carrying One or Two t Haplotypes Are Deficient in Investment and Oocyte Penetration. Dev. Biol. 168:138–49.

Jones, R. C. 1999. To store or mature spermatozoa? The primary role of the epididymis. Int. J. Androl. 22:57–67.

Kettaneh, N. P., and D. L. Hartl. 1980. Ultrastructural analysis of spermiogenesis in segregation distorter males of Drosophila melanogaster: The homozygotes. Genetics 96:665–683.

Lee, M. R., and F. F. Elder. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenet. Cell Genet. 26:36–40.

Long, T. a F., and A. Pischedda. 2005. Do female Drosophila melanogaster adaptively bias offspring sex ratios in relation to the age of their mate? Proc. Biol. Sci. 272:1781–1787.

Lowe, X., S. O'Hogan, D. M. II, J. Bishop, and A. Wyrobek. 1996. Aneuploid epididymal sperm detected in chromosomally normal and Robertsonian translocation-bearing mice using a new three-chromosome FISH method. Chromosoma 204–210.

Mange, A. 1970. Possible nonrandom utilization of X-and Y-bearing sperm in Drosophila melanogaster. Genetics 65:95–106.

Newton, M. E., R. J. Wood, and D. I. Southern. 1976. A cytogenetic analysis of meiotic drive in the mosquito, Aedes aegypti (L.). Genetica 46:297–318.

Olds-Clarke, P. 1996. How does poor motility alter sperm fertilizing ability? J. Androl. 17:183–186.

Olds-Clarke, P., and L. R. Johnson. 1993. T Haplotypes in the Mouse Compromise Sperm Flagellar Function.

Price, T. a R., D. J. Hodgson, Z. Lewis, G. D. D. Hurst, and N. Wedell. 2008. Selfish genetic elements promote polyandry in a fly. Science 322:1241–1243.

Reinhardt, J. a, C. L. Brand, K. a Paczolt, P. M. Johns, R. H. Baker, and G. S. Wilkinson. 2014. Meiotic drive impacts expression and evolution of x-linked genes in stalk-eyed flies. PLoS Genet. 10:e1004362.

Rice, W. R. 2014. An X-Linked Sex Ratio Distorter in Drosophila simulans That Kills or Incapacitates Both Noncarrier Sperm and Sons. G3 (Bethesda). 4:1837–48.

Rice, W. R., S. Gavrilets, and U. Friberg. 2008. Sexually antagonistic "zygotic drive" of the sex chromosomes. PLoS Genet. 4:e1000313.

Saunders, P. A., J. Perez, M. Rahmoun, O. Ronce, P.-A. Crochet, and F. Veyrunes. 2014. XY females do better than the XX in the african pygmy mouse, Mus minutoides. Evolution (N. Y). 68:2119–27.

Suarez, S. 2008. Regulation of sperm storage and movement in the mammalian oviduct. Int J Dev Biol 462:455–462.

Sweeny, T. L., and a R. Barr. 1978. Sex Ratio Distortion Caused By Meiotic Drive. Genetics 88:427–46.

Taylor, D. R., and P. K. Ingvarsson. 2003. Common features of segregation distortion in plants and animals. Genetica 117:27–35.

Veyrunes, F., P. Chevret, J. Catalan, R. Castiglia, J. Watson, G. Dobigny, T. J. Robinson, and J. Britton-Davidian. 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. B 277:1049–56.

Ward, M. A., and P. S. Burgoyne. 2006. The Effects of Deletions of the Mouse Y Chromosome Long Arm on Sperm Function — Intracytoplasmic Sperm Injection (ICSI) -Based Analysis. Biol. Reprod. 74:652–658.

Weichenhan, D., W. Traut, B. Kunze, and H. Winking. 1996. Distortion of Mendelian recovery ratio for a mouse HSR is caused by maternal and zygotic effects. Genet. Res. 68:125–9.

Werren, J. H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. Proc. Natl. Acad. Sci. U. S. A. 108:10863–10870.

Werren, J. H., and L. W. Beukeboom. 1998. Sex Determination, Sex Ratios, and Genetic Conflict. Annu. Rev. Ecol. Sys. 29:233–261.

Wilkinson, G. S., and M. I. Sanchez. 2001. Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, Cyrtodiopsis whitei. Heredity (Edinb). 87:17–24.

SUPPLEMENTARY MATERIAL

Table S1. X and Y ratio of metaphase II spermatocytes, Count table.

Male ID	N	Y count	X count	NA	Y-ratio	P-value
K8879	51	25	26	0	0.49	1
K8880	50	22	20	8	0.52	0.88
K8876	50	20	18	12	0.53	0.87
K8877	50	19	19	12	0.5	1
K8878	54	22	19	13	0.54	0.75
K8804	51	21	24	6	0.46	0.76

N: number of metaphases inspected, *NA*: ambiguous sex chromosome, *p-value*: from a binomial test.

Table S2. X and Y ratio of mature sperm, count table.

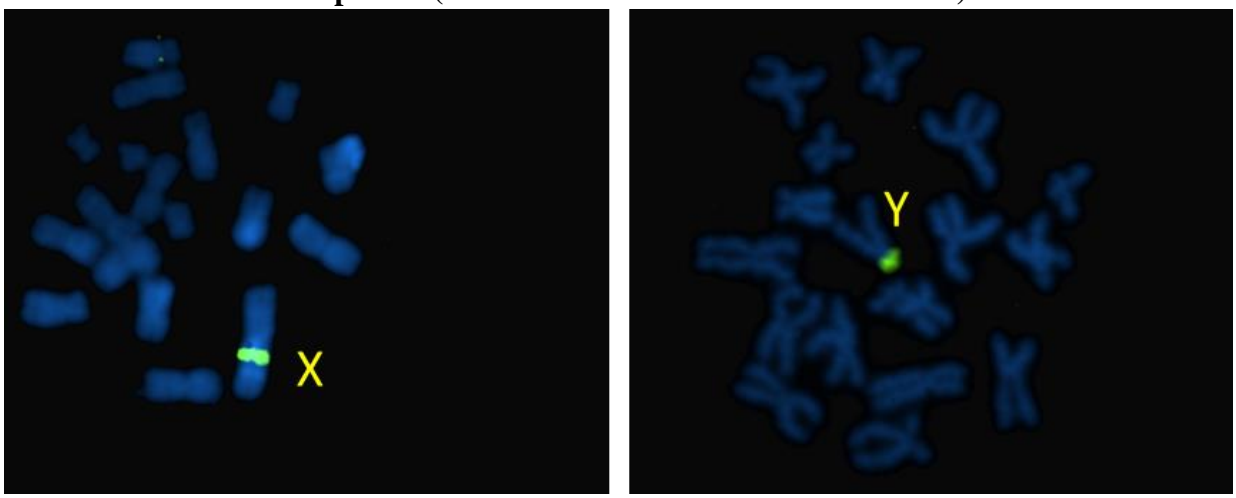
Male ID	Partner's genotype	Y count	X count	NA	Y-ratio	P-value
63.2.2M	XX*	100	106	1	0.49	0.73
88.6.6M	XX	115	106	10	0.52	0.59
117.6.3M	XX	107	105	4	0.50	0.95
180.4.1M	X*Y	102	95	12	0.52	0.67
77.6.3M	XX*	94	99	NA	0.49	0.77

NA: no signal or ambiguous signal, *p-value* from a binomial test.

Table S3. Genotyped embryo count

Embryo genotype	XX* (32 females)	X*Y (30 females)
NA	26	72
XY	52	59
XX	16	-
XX*	13	58
X*Y	44	29
XX or XX*	13	-
XX* or X*Y	0	2
XY or X*Y	5	3
total	169	224

Figure S1. BAC-mapping of mouse BACs CH29 168-N4 (X) and CH29 604-J10 (Y) on *Mus minutoides* metaphases (both stained with CY3 fluorochromes).



CHAPITRE 2 : les conséquences de l'évolution du système de déterminisme du sexe atypique de *Mus minutoides*

Manuscrit 4: “Anatomical and molecular analyses of XY ovaries from the African pygmy mouse *Mus minutoides*”

Chez les mammifères, une modification dans la cascade génétique du déterminisme du sexe mène généralement à des problèmes de différentiation sexuelle qui se traduisent par des caractères sexuels primaires et secondaires ambigus plus ou moins prononcés si la mutation est très en amont ou en aval de la cascade (Eggers and Sinclair 2012; Jiménez et al. 2013). Les femelles X*Y chez *Mus minutoides* sont indistinguable des autres types de femelles au sein de l'élevage, et nous nous sommes posés la question de savoir si la présence du chromosome Y chez ces femelle affectait leur caractères anatomiques et morphologiques, en analysant les organes génitaux externes et internes des mâles et des trois types de femelles.[6]

Malgré le fait que le chromosome Y soit fonctionnel chez les femelles X*Y (le gène *Sry* est transcrit dans leur cerveau et leurs gonades à l'âge adulte), l'inversion du sexe de ces femelles est complète. Elles présentent des ovaires identiques à ceux des autres femelles, exprimant les gènes marqueurs des tissus ovariens *Foxl2* et *Wnt4*, sans aucune trace de différenciation testiculaire (renseignant sur le fait que la mutation responsable de l'inversion du sexe sur le X* doit affecter la cascade du déterminisme du sexe très précocemment, et que le gène impliqué est sans doute un acteur proche de *Sry*). D'un point de vue morphologique, elles ont en moyenne un poids et une distance ano-genitale (marqueur classique de dimorphisme sexuel, reflétant le niveau d'androgènes circulant) identiques à ceux des femelles XX et XX*.

Les femelles X*Y sont donc des femelles comme les autres en ce qui concerne les caractères sexuels primaires et la morphologie. Cependant, il est possible qu'elles se distinguent des femelles XX et XX* concernant d'autres caractères sexuels secondaires. La mise en évidence de la présence de transcrits de *Sry* dans le cerveau des femelles X*Y (gène est impliqué dans la différentiation sexuelle du cerveau, Dewing et al. 2006; Sekido 2014) pourrait suggérer qu'elles puissent par exemple avoir des comportements masculinisés.

[6] Ma contribution à cette étude concerne l'étude des organes génitaux externes, et les mesures morphologiques.

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Anatomical and Molecular Analyses of XY Ovaries from the African Pygmy Mouse *Mus minutoides*

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Key Words
FOXL2 · Sex reversal · SOX9 · SRY

Abstract
The African pygmy mouse *Mus minutoides* is characterized by the presence of a high proportion of fertile XY females in natural populations. This species displays 2 morphologically different X chromosomes: the ancestral X and a shorter one designated as X*, feminizing the X*Y individuals. This strongly suggests that in the presence of an X* chromosome, the male differentiation program is not activated despite a functional Y chromosome. In this study, we compared the histology of the adult ovaries of the 3 female genotypes (XX, XX* and X*Y) and investigated the expression of some of the main genes involved in male and female differentiation. We found that X*Y gonads display a typical ovarian structure without any testicular organization. Moreover, the ovarian somatic marker FOXL2 is detected in X*Y follicle cells and exhibits the same pattern as in XX and XX* ovaries, whereas SOX9 and DMRT1 are absent at all stages of follicular differentiation. However, surprisingly, X*Y ovaries display a higher level of *Sry* transcripts compared to testes. Our findings confirm the complete sex reversal in X*Y individuals with no apparent sign of masculinization, providing an attractive model to unravel new gene interactions involved in the mammalian sex determination system.

Sex is established during early development, and various mechanisms, depending on the species, are involved in this sex determination process. In species with heteromorphic sex chromosomes, such as birds and mammals, sex is fixed at fertilization by the differential inheritance of sex chromosomes. In most mammals, XX embryos are destined to become females, while XY embryos will develop into males. Mechanistically, the Y chromosome-linked *Sry* gene [Sinclair et al., 1990] initiates a cascade of male-specific gene expression in the undifferentiated gonads of XY embryos, resulting in testicular differentiation (for a re-

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view, see Wainwright and Wilhelm [2010]). The main role of the SRY protein is the testis-specific activation of the *Sox9* gene [Sekido and Lovell-Badge, 2009], which encodes a transcription factor of the SOX family inducing the differentiation of the male-supporting cell lineage, the Sertoli cells. In the absence of *Sry/Sox9* expression, the forkhead/winged helix transcription factor FOXL2 and the R-spondin1/WNT4/β-catenin signaling pathway act independently but complementarily to promote and maintain ovarian development while suppressing testis development [Chassot et al., 2008; Wilhelm et al., 2009].

This system is finely regulated and any deviation generally leads to gonadal malformation or dysfunction. Indeed, several cases of sex reversal and intersexuality have been reported and are commonly associated with sterility (for a review, see Quinn and Koopman [2012]; Warr and Greenfield [2012]). In several species of rodents, however, natural but unusual sex chromosome mechanisms evolved without affecting fertility. For instance, in the spiny rats *Tokudaia osimensis* and *T. tokunoshimensis,* both males and females are X0, with males having lost their Y chromosome along with their *Sry* gene [Soullier et al., 1998; Kuroiwa et al., 2010]. Additional copies of *Cbx2*, a gene acting upstream of *Sry* [Katoh-Fukui et al., 1998], were identified in males, suggesting that CBX2 might be involved in male sex determination in *Tokudaia* species [Kuroiwa et al., 2011]. Another remarkable pattern, in the opposite sense of that described above, is exhibited by different species of South American field mice of the genus *Akodon* and the lemmings *Myopus schisticolor* and *Dicrostonyx torquatus*, in which females are XX, XX* or X*Y. In these 3 taxa, the X* differs from the X by independent chromosomal rearrangements, but these probably involve the same region [Herbst et al., 1978; Fredga, 1988; Ortiz et al., 2009]. In X*Y individuals, no testis develops despite the presence of a normal Y chromosome and an unaltered *Sry* [Sánchez and Vilain, 2010].

An additional case of fertile XY females has been recently described in the African pygmy mouse, *Mus minutoides* [Veyrunes et al., 2010]. Natural populations of this species are characterized by a very high proportion (up to 80%) of fully fertile XY females phenotypically indistinguishable from females of other genotypes [Veyrunes et al., 2013]. As in lemmings and *Akodon*, molecular and karyological analyses revealed the existence of 2 morphologically different X chromosomes: the ancestral X and a rearranged X* [Veyrunes et al., 2010]. Again, the sex reversal in *M. minutoides* is not due to a deficiency of the *Sry* gene nor to a deficiency of the Y chromosome, but to a still unknown X*-linked mutation.

These particular and independent sex determination systems described in *Akodon*, lemmings and *M. minutoides* suggest an important contribution of the X chromosome in the achievement of the male sex-determining program controlled by *Sry*. In addition, *M. minutoides* being a close relative of *M. musculus*, this model strongly benefits from the knowledge and molecular tools developed for the laboratory mouse. Most of these resources are thus easily transferable to the African pygmy mouse.

In this study, we address the question of whether the Y chromosome in *M. minutoides* X*Y females perturbs the correct organization of the adult ovary. For this, we examined the external and internal genitalia of males and females, the gonad histology, and we analyzed the expression of key sex-specific genes *(Foxl2*, *Sox9, Wnt4* and *Dmrt1)* at the transcriptional and/or at the protein level in X*Y *M. minutoides* adult ovaries. We found that X*Y gonads display a typical ovarian structure without any detectable testicular organization. Moreover, the ovarian granulosa cell marker FOXL2 was detected in X*Y follicles and exhibits the same pattern as in XX and XX* ovaries. In contrast, SOX9 was not found in granulosa cells at any stage of follicular differentiation. Interestingly, we detected the *Sry* transcript in adult X*Y ovary. Altogether, these findings confirm the complete sex reversal in X*Y individuals with no apparent sign of masculinization.

Materials and Methods

Animals and Dissection

M. minutoides mice were bred from our own laboratory colony established in June 2010 from 13 animals caught in the Caledon Nature Reserve, South Africa. Males and females of each genotype (XX, XX* and X*Y) were used in this study. The female's genotype was systematically assessed by karyotyping based on chromosome preparations from bone marrow of yeast-stimulated animals [Lee and Elder, 1980] and/or PCR amplification of the Y-specific *Sry* gene, following the procedure already described [Veyrunes et al., 2010]. Five- to six-month-old animals were sacrificed by cervical dislocation. For the analysis of internal genitalia, reproductive tracts were dissected in ice-cold PBS, and pictures were taken through a Leica MZ6 binocular microscope with a Nikon F300 camera. For histological analysis and immunofluorescence experiments, dissected ovaries and testes were fixed in 4% paraformaldehyde in PBS. For RNA isolation, dissected brains and gonads were frozen on dry ice and stored at –80°C until further processing.

Mice were housed according to international standard conditions, and all animal experiments were performed in accordance with European guidelines and with the approval of the Ethical Committee on animal care and use of France (No. CEEA-LR-12170).

Table 1. Primer sequences for real-time PCR

Gene	Primer	
	Forward	Reverse
Foxl2	5′-ACAACCTCAGCCTCAACGAG-3′	5′-TCGAGCGTCCAGTAGTTGC-3′
Rps29	5′-TGAAGGCAAGATGGGTCAC-3′	5′-GCACATGTTCAGCCCGTATT-3′
Sox9	5′-TCGGACACGGAGAACACC-3′	5′-GCACACGGGGAACTTATCTT-3′
Sry	5′-AAAAGCCTTACAGAAGACGAAAAA-3′	5′-TCTCTGTGTAGGGTCTTCAGTCTC-3′
Wnt4	5′-GCGTAGCCTTCTCACAGTCC-3′	5′-CGCATGTGTGTCAAGATGG-3′

Identification of the Estrous Stages

When the ovaries were collected, the estrous cycle stage of the female was determined: vaginal smears were hematoxylin-eosin stained and analyzed with a light microscope (BH2, Olympus). Qualification and quantification of cell types were performed at 10× magnification. The diestrous phase was defined by the exclusive presence of leukocytes, the proestrous phase by leukocytes and nucleated epithelial cells, the estrous phase by large and squamous type epithelial cells without nuclei, and metestrous by leukocytes and epithelial cells with translucent nuclei.

Anogenital Distance Measurements

Anogenital distance (AGD) is a sexually dimorphic trait in mice and defined as the distance between the anterior end of the anus and the posterior end of the genital papilla [Marty et al., 2003]. It is a marker of circulating androgen [Drake et al., 2009; Eisenberg et al., 2011]. The AGD of 23 males and 31 females of each genotype was measured using a digital caliper with a 0.01-mm accuracy under a binocular microscope. Two measurements were made on each individual and the averaged values were used. The effect of female genotype on AGD was assessed using an analysis of covariance including body mass (measured at the nearest 0.01 g using a digital scale) as a covariate.

Histological Analysis and Immunofluorescence Staining

After fixation, adult gonads were processed and embedded in paraffin. Three-μm sections were stained with hematoxylin and eosin for histological analysis or processed for immunofluorescence. For the latter, sections were incubated with the following primary antibodies: mouse anti-DDX4/MVH antibody (1:1,500 dilution) (Abcam) to mark germ cells; rabbit anti-FOXL2 (1:100 dilution), generated as described in Cocquet et al. [2002], rabbit anti-SOX9 Cter (1:300 dilution) raised against the TA domain of human SOX9 [De Santa Barbara et al., 1998], and rabbit anti-DMRT1 (1:100 dilution), a kind gift of D. Zarkower [Raymond et al., 2000]. Secondary antibodies (1:800 dilution) used were: donkey anti-rabbit Alexa Fluor 488 and donkey anti-rabbit Alexa Fluor 555 (Invitrogen Molecular Probes). Slides were subsequently stained with Hoechst 33342 (Sigma Aldrich) and mounted with FluorSave reagent (Calbiochem). External and internal reproductive organs were imaged with a digital camera (Nikon). Hematoxylin- and eosin-stained sections were imaged using a HT 2.0 Hamamatsu nanozoomer. Immunofluorescence pictures were obtained with a Leica DM6000 fluorescent microscope. All images were processed with Adobe Photoshop software.

RNA Isolation, Reverse Transcription and Quantitative Real-Time RT-PCR

Total RNA from adult gonads and brain was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. Reverse transcription was carried out with 0.5–2 μg of DNase-treated total RNA (Applied Biosystems) and converted to 1st-strand cDNA using SuperScript III Reverse Transcriptase following the manufacturer's instructions (Invitrogen Life Technologies).

Quantitative real-time (qRT) PCR was carried out in a LightCycler 480 System (Roche) by using the LightCycler 480 SYBR Green I Master Mix (Roche). Expression levels of *Foxl2*, *Sox9*, *Wnt4*, *Sry*, and *Rps29* genes were measured in triplicates in at least 3 separate assays. Primers used for qRT-PCR are listed in table 1 and were designed using the Universal Probe Library software (Roche) based on the *M. musculus* transcript sequences. PCR products obtained from *M. minutoides* cDNA were cloned and sequenced subsequently to confirm identity. Quantification was performed using a second derivative calculation method provided by LC480 software version 1.5 (Roche) and *Rps29* was used as the reference gene [Svingen et al., 2009]. Statistical analysis was performed using Mann-Whitney test (Prism software) and results were considered statistically significant at $p < 0.05$.

Results and Discussion

*Complete Sex-Reversed Phenotype of X*Y Females*

The external and internal reproductive organs of adult X*Y female mice (fig. 1A, E) were indistinguishable from those of XX* and XX females (fig. 1B, F, and C, G, respectively), whereas they were clearly different from XY males (fig. 1D, H). In particular, AGD is similar in the 3 female genotypes [$F_{2,90} = 0.15$, $p = 0.86$; no influence of body mass was found on female AGD ($F_{1,89} = 1.61$, $p = 0.21$)] and much shorter than in males ($F_{1,110} = 902.05$, $p < 0.001$) (fig. 1A–D; table 2), therefore suggesting an equivalent low level of circulating androgen in females [Eisenberg et al., 2011].

A normal uterine structure was observed in X*Y females identical to the uterus in XX* and XX (fig. 1E–G),

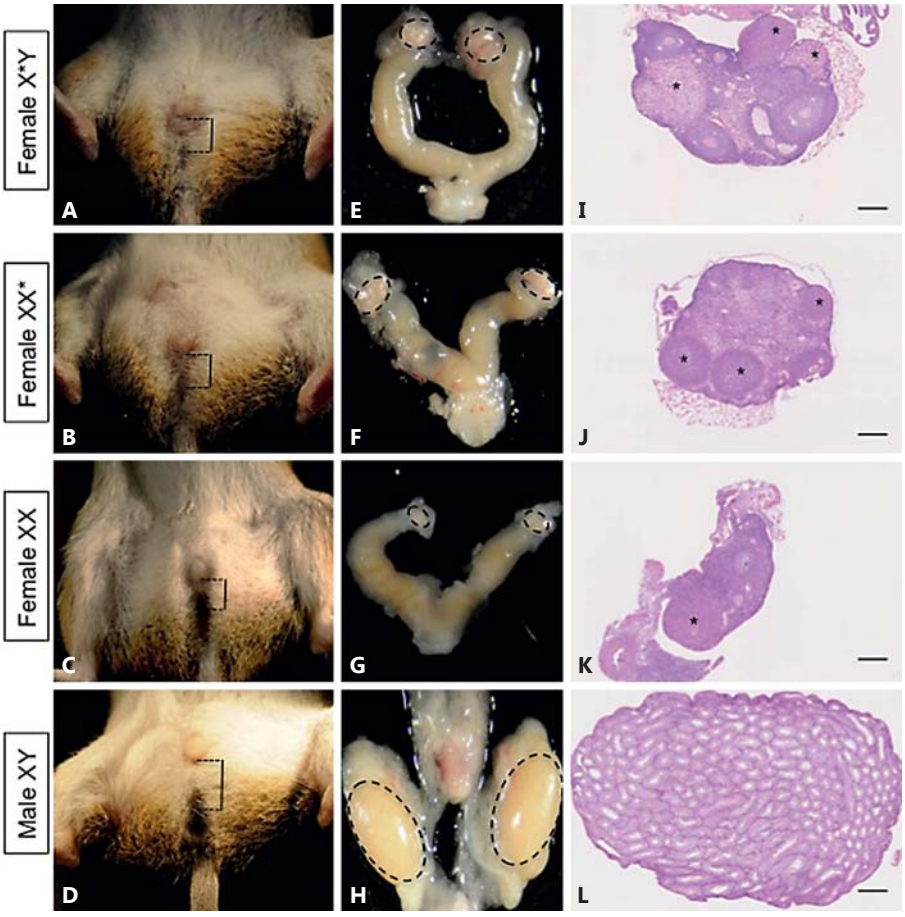

Fig. 1. Complete sex-reversed phenotype of X*Y *M. minutoides* mice. **A–D** External genitals. **E–H** Internal reproductive organs, and **I–L** hematoxylin and eosin (H&E)-stained gonad sections from adult *M. minutoides* mice. External and internal reproductive organs from X*Y female mice (**A**, **E**) are morphologically indistinguishable from those of other female genotypes XX* (**B**, **F**) and XX (**C**, **G**). The AGD indicated by black lines (**A–D**) shows no difference between females whereas it is larger in males. Dotted circles point out the ovaries (**E–G**) and testes (**H**). H&E-stained ovaries from X*Y females (**I**) appear normal and contain follicles at different maturation stages, including antral follicles with oocytes and corpora lutea (indicated by asterisks) similar to XX* (**J**) and XX (**K**) females. X*Y females do not exhibit any external (**D**) or internal (**H**) male genitalia, or testicular tissue (**L**). Magnification: 1× (**A–D**) and 2× (**E–H**). Scale bars represent 50 μm.

suggesting that no anti-Müllerian hormone secretion occurred during fetal life of sex-reversed females [di Clemente et al., 1994]. In addition, Wolffian ducts were also completely undeveloped in X*Y, XX* and XX females. Taken together, these observations strongly suggested that no testicular hormones have been secreted by X*Y gonads during fetal life.

Histological analysis of X*Y and XX* ovaries revealed no obvious abnormalities (fig. 1I–K) and showed that gonads from these females display a typical ovary structure with no apparent sign of testicular differentiation. Hematoxylin- and eosin-stained ovaries from X*Y females had oocytes and follicles at different developmental stages and many corpora lutea (fig. 1I) indicating that ovulation took place in these females and making their ovaries indistinguishable from XX* ovaries. However, it is noteworthy that XX mice (fig. 1K) had smaller ovaries than X*Y and XX* females with less follicles at a comparable age and estrous stage (here estrus) (fig. 1I and J, respectively). This observation may explain the lower breeding performances of XX females compared to the X*Y [Saunders et al., 2014]. However, the fact that the XX* females have large ovaries but comparable breeding performances to the XX rules out any causal link between ovary size and reproductive output. A possible explanation for the histological features of the XX ovaries is that a recessive mutation on the X chromosome, rare in natural populations, might have been fixed in the laboratory colony due to the small number of founder individuals. Hence, this result may not reflect the exact conditions in wild popula-

Table 2. The average AGD and body mass of males and females

Genotype	Animals, n	AGD ± SD, mm	Body mass ± SD, g
XY	23	7.28±1.14	9.02±2.4
XX	31	2.34±0.46	7.41±1.52
XX*	31	2.40±0.49	7.59±1.61
X*Y	31	2.36±0.45	7.31±1.63

tions and could be clarified by additional ovarian histological analyses of wild-caught specimens. Interestingly, sex reversal in *M. minutoides* does not correspond to a pathological condition alike the XX hermaphroditism found in female moles of the genus *Talpa*. In these species, testicular tissue develops in the absence of a Y chromosome, and XX females have ovotestes, in which the ovarian region contains normal follicles, representing the fertile component of the gonad, whereas the testicular tissue is dysgenic but occupies most of the gonadal volume [Jiménez et al., 1993; Barrionuevo et al., 2004].

*Expression of Key Ovary (Foxl2, Wnt4) and Testis (Sox9 and Dmrt1) Genes in X*Y Females*

Since X*Y mice are phenotypically female and fertile, it suggests that the male differentiation program is not functional in these females. To test this hypothesis, we investigated the expression of SOX9 and DMRT1 in adult ovaries of X*Y and of the 2 other female genotypes (even in the absence of apparent testicular tissue) as well as the expression of FOXL2, which acts antagonistically to SOX9 [Wilhelm et al., 2009] and to DMRT1 [Uhlenhaut et al., 2009].

The granulosa marker FOXL2 was detected in X*Y follicles (fig. 2A, B) and exhibited the same expression pattern, i.e. in granulosa cells at all stages of follicular differentiation, as in XX and XX* ovaries (fig. 2C, D and E, F, respectively). In XX ovaries, FOXL2 was expressed despite the low follicle number. In contrast, SOX9 was absent in granulosa cells of X*Y and XX* ovaries (fig. 2G, H and K, L, respectively) and XX ovaries (not shown), whereas SOX9 was expressed in the supporting cells of the testicular tubules (fig. 2I, J), validating the recognition of the *M. minutoides* SOX9 protein by our antibody. Similarly, DMRT1 protein is not found in any ovarian compartment of X*Y and XX* females (fig. 2M, N and Q, R, respectively), while it is detected in Sertoli cells of XY mice (fig. 2O, P). Hence, our immunofluorescence experiments indicated proper establishment of the ovarian pathway in adult X*Y gonads without the expression of the Sertoli cell marker SOX9 nor of the male gonadal regulator DMRT1, thus confirming the complete male-to-female sex reversal phenotype observed in these mice.

In order to correlate FOXL2 and SOX9 proteins to their respective transcript levels, we performed a qRT-PCR on total RNA obtained from ovaries of 4 females of each genotype and testes from 4 males. As expected, *FoxL2* mRNA was expressed at similar levels in adult ovaries of all 3 genotypes, while *Sox9* transcripts were also detected in the 3 females genotypes (fig. 3), which is con-

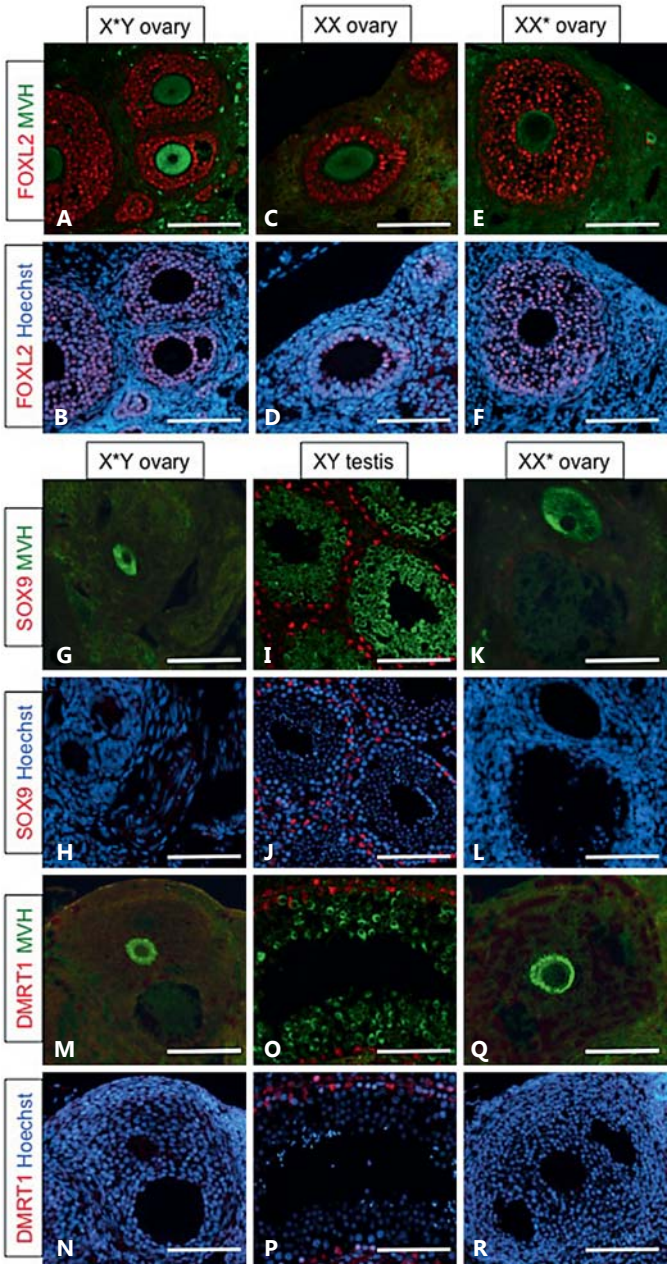

Fig. 2. Analysis of FOXL2, SOX9 and DMRT1 expression in adult X*Y female gonads. **A–F** Fluorescence microscopy of paraffin sections of X*Y and control females (XX and XX*) double immunostained for FOXL2 (red, nuclear) and MVH (green, cytoplasmic). FOXL2 appears to be normally expressed in granulosa cells of ovaries of all 3 female genotypes with a nuclear localization as revealed by Hoechst staining (**B**, **D**, **F**). **G–R** Paraffin sections of X*Y and control mice (male and XX* female) double immunolabeled for SOX9 (red, nuclear) and MVH (green, cytoplasmic), and DMRT1 (red, nuclear) and MVH (green, cytoplasmic). SOX9 and DMRT1 proteins are detected in the nuclei of Sertoli cells in testis (**I**, **J** and **O**, **P**, respectively), but not in granulosa cells of the different females (**G**, **H**, **K**, **L**, **M**, **N**, **Q**, **R**). As expected, MVH protein is present in the germ cell compartment of both female and male gonads. Scale bars represent 100 μm.

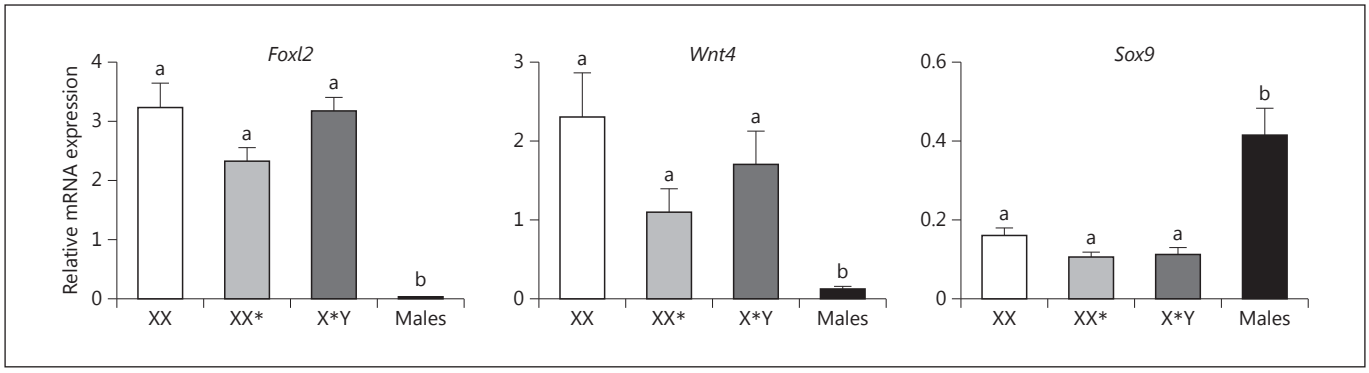

Fig. 3. Quantification of *Foxl2*, *Wnt4* and *Sox9* expression in adult X*Y ovaries normalized to *Rps29* total RNA levels. X*Y gonads express *Foxl2*, *Wnt4* and *Sox9* transcripts at similar levels to XX* and XX ovaries. As expected, control male gonads do not express either *Foxl2* or *Wnt4*, but show higher levels of *Sox9* compared to ovaries. Each histogram corresponds to 4 mice. Data represent the mean ± SEM values of triplicates from at least 3 separate experiments. The different letters above the histograms indicate significant differences according to Mann-Whitney test ($p < 0.05$).

sistent with a previous report in *M. musculus* [Notarnicola et al., 2006]. In adult testes, the *FoxL2* transcript was found to be almost undetectable and *Sox9* mRNA level to be 2.5-fold higher than in ovaries (fig. 3).

Other factors that have been shown to impact ovarian cell function are members of the Wnt family. As *Wnt4*, in particular, has been found to be involved in sexual differentiation by suppressing male sexual differentiation, promoting Müllerian duct differentiation and maintaining oocyte health [Vainio et al., 1999], we determined its expression in X*Y ovaries. RT-PCR analyses revealed that *Wnt4* mRNA levels are similar in adult ovaries of all 3 genotypes, being comparable to the *Foxl2* profile (fig. 3). On the contrary, *Wnt4* expression is markedly reduced in *M. minutoides* testes, which is in agreement with the reported *Wnt4* profile in *M. musculus* adult gonads (http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4503:1450782_at).

*Sry Expression in Adult X*Y Females*

A potential cause of the sex reversal observed in X*Y female could be a defect in the regulation of *Sry* expression. In *M. musculus*, mutations in genes involved in the regulation of *Sry* expression induce sex-reversed phenotypes as described for *Cbx2* [Katoh-Fukui et al., 1998], *Wt1* [Hammes et al., 2001; Bradford et al., 2009] and, more recently, for *Jmjd1a* [Kuroki et al., 2013].

Therefore, we first investigated *Sry* expression in adult brain and found that *Sry* transcripts were expressed in X*Y female brains at a similar level to male brains, while no transcripts were detected in XX* control females (fig. 4A), showing that all factors required for expression of the *Sry* transcript in brain were present in X*Y females. Moreover, *Sry* is reported to be highly expressed in dopaminergic-neuron rich regions of the male mammalian brain, where it maintains their biochemical and motor function without any mediation by gonadal hormones [Dewing et al., 2006]. Here, X*Y individuals display ovaries, a female testosterone level and would express SRY in their brain as in males. The coexistence of these parameters in the same animal constitutes a very interesting context to elucidate in the future, namely the role of SRY protein in such neurons.

Furthermore, we explored *Sry* expression in *M. minutoides* adult gonads. Surprisingly, adult X*Y ovaries displayed a higher level of *Sry* transcript compared to testes, while no transcripts were detected in XX* and XX ovaries as expected (fig. 4B). In the adult testis of *M. musculus,* a circular *Sry* transcript is expressed in round spermatid but not in testicular somatic cells [Capel et al., 1993; Hendriksen et al., 1995], whereas in adult mouse brain, the *Sry* transcript is linear [Lahr et al., 1995]. Only linear transcripts are apparently functional and are translated into protein [Capel et al., 1993]. So far, we cannot rule out the existence of *Sry* circular transcripts in adult *M. minutoides* X*Y ovaries. Hence, to investigate if SRY protein is expressed in adult X*Y ovaries, we used the only antibody efficient in immunofluorescence experiments for mouse SRY [Bradford et al., 2007]. Unfortunately, the antibody raised against *M. musculus* SRY did not recognize *M. minutoides* SRY protein, probably due to its shorter C-terminal domain [unpubl. data]. The development of an

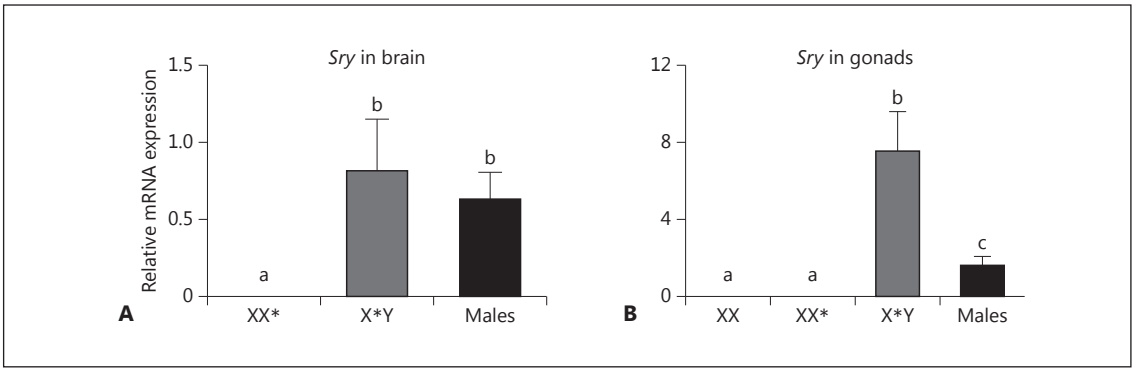

Fig. 4. Quantification of *Sry* transcript levels in adult X*Y tissues normalized to *Rps29* total RNA levels. Relative expression levels of *Sry* total RNA in X*Y, XX* female and XY male adult brains (**A**), and in X*Y, XX*, XX female and XY male adult gonads (**B**). Results show a similar level of *Sry* expression in X*Y female and XY male brains, and a higher level in X*Y female ovaries than in XY male testes. As expected, *Sry* expression was not detectable in other females. Each histogram corresponds to 4 mice. Data represent the mean ± SEM values of 3 separate experiments performed in triplicate. The different letters above the histograms indicate significant differences according to Mann-Whitney test ($p < 0.05$).

antibody that specifically detects *M. minutoides* SRY is clearly needed.

In summary, our investigation showed that even in the presence of a functional Y chromosome, the complex chromosomal rearrangement changing the X chromosome into the derivative X* allows the formation of a fully fertile ovary with no apparent sign of masculinization in X*Y *M. minutoides* individuals. This suggests a strong influence of the X chromosome on the male sex-determining program controlled by *Sry*. In human patients with disorders of sex development, the X-linked gene *DAX1* has been described to induce male-to-female sex reversal when duplicated [Swain et al., 1996]. However, in the case of *M. minutoides*, experiments of comparative genomic hybridization array and quantitative PCR on genomic DNA as well as on qRT-PCR did not provide any evidence of rearrangement or transcriptional upregulation of the *Dax1* gene from the X* chromosome [unpubl. data], arguing for the involvement of other unknown genetic determinants located on the X chromosome, but absent or altered on the X* chromosome. Moreover, because this particular sex-determining system using a derivative X* chromosome also evolved independently in other species such as lemmings and *Akodon* [Fredga, 1988; Ortiz et al., 2009], it suggests that the mammalian X chromosome carries at least one gene as important as *Sry* for the testis-determining pathway. Therefore, *M. minutoides* constitutes an original model that may provide valuable insights into the complex mammalian sex differentiation cascade and human disorders of sex development.

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References

Barrionuevo FJ, Zurita F, Burgos M, Jiménez R: Testis-like development of gonads in female moles. New insights on mammalian gonad organogenesis. Dev Biol 268:39–52 (2004).

Bradford ST, Wilhelm D, Koopman P: Comparative analysis of anti-mouse SRY antibodies. Sex Dev 1:305–310 (2007).

Bradford ST, Wilhelm D, Bandiera R, Vidal V, Schedl A, Koopman P: A cell-autonomous role for *Wt1* in regulating *Sry* in vivo. Hum Mol Genet 18:3429–3438 (2009).

Capel B, Swain A, Nicolis S, Hacker A, Walter M, et al: Circular transcripts of the testis-determining gene *Sry* in adult mouse testis. Cell 73: 1019–1030 (1993).

Chassot AA, Ranc F, Gregoire EP, Roepers-Gajadien HL, Taketo MM, et al: Activation of β-catenin signaling by *Rspo1* controls differentiation of the mammalian ovary. Hum Mol Genet 17:1264–1277 (2008).
Cocquet J, Pailhoux E, Jaubert F, Servel N, Xia X, et al: Evolution and expression of *Foxl2*. J Med Genet 39:916–921 (2002).
De Santa Barbara P, Bonneaud N, Boizet B, Desclozeaux M, Moniot B, et al: Direct interaction of Sry-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. Mol Cell Biol 18:6653–6665 (1998).
Dewing P, Chiang CW, Sinchak K, Sim H, Fernagut PO, et al: Direct regulation of adult brain function by the male-specific factor SRY. Curr Biol 16:415–420 (2006).
di Clemente N, Wilson C, Faure E, Boussin L, Carmillo P, et al: Cloning, expression, and alternative splicing of the receptor for anti-Müllerian hormone. Mol Endocrinol 8:1006–1020 (1994).
Drake AJ, van den Driesche S, Scott HM, Hutchison GR, Seckl JR, Sharpe RM: Glucocorticoids amplify dibutyl phthalate-induced disruption of testosterone production and male reproductive development. Endocrinology 150: 5055–5064 (2009).
Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI: The relationship between anogenital distance, fatherhood, and fertility in adult men. PLoS One 6:e18973 (2011).
Fredga K: Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. Philos Trans R Soc Lond B Biol Sci 322:83–95 (1988).
Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, et al: Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell 106:319–329 (2001).
Hendriksen PJ, Hoogerbrugge JW, Themmen AP, Koken MH, Hoeijmakers JH, et al: Postmeiotic transcription of X and Y chromosomal genes during spermatogenesis in the mouse. Dev Biol 170:730–733 (1995).
Herbst EW, Fredga K, Frank F, Winking H, Gropp A: Cytological identification of 2 X-chromosome types in wood lemming *(Myopus schisticolor)*. Chromosoma 69:185–191 (1978).
Jiménez R, Burgos M, Sánchez A, Sinclair AH, Alarcón FJ, et al: Fertile females of the mole *Talpa occidentalis* are phenotypic intersexes with ovotestes. Development 118:1303–1311 (1993).
Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, et al: Male-to-female sex reversal in *M33* mutant mice. Nature 393: 688–692 (1998).
Kuroiwa A, Ishiguchi Y, Yamada F, Shintaro A, Matsuda Y: The process of a Y-loss event in an X0/X0 mammal, the Ryukyu spiny rat. Chromosoma 119:519–526 (2010).
Kuroiwa A, Handa S, Nishiyama C, Chiba E, Yamada F, et al: Additional copies of *CBX2* in the genomes of males of mammals lacking *Sry*, the Amami spiny rat *(Tokudaia osimensis)* and the Tokunoshima spiny rat *(Tokudaia tokunoshimensis)*. Chromosome Res 19:635–644 (2011).
Kuroki S, Matoba S, Akiyoshi M, Matsumura Y, Miyachi H, et al: Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a. Science 341:1106–1109 (2013).
Lahr G, Maxson SC, Mayer A, Just W, Pilgrim C, Reisert I: Transcription of the Y chromosomal gene, *Sry*, in adult mouse brain. Brain Res Mol Brain Res 33:179–182 (1995).
Lee MR, Elder FF: Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenet Cell Genet 26:36–40 (1980).
Marty MS, Chapin RE, Parks LG, Thorsrud BA: Development and maturation of the male reproductive system. Birth Defects Res B Dev Reprod Toxicol 68:125–136 (2003).
Notarnicola C, Malki S, Berta P, Poulat F, Boizet-Bonhoure B: Transient expression of SOX9 protein during follicular development in the adult mouse ovary. Gene Expr Patterns 6: 695–702 (2006).
Ortiz MI, Pinna-Senn E, Dalmasso G, Lisanti JA: Chromosomal aspects and inheritance of the XY female condition in *Akodon azarae* (Rodentia, Sigmodontinae). Mamm Biol 74:125–129 (2009).
Quinn A, Koopman P: The molecular genetics of sex determination and sex reversal in mammals. Semin Reprod Med 30:351–363 (2012).
Raymond CS, Murphy MW, O'Sullivan MG, Bardwell VJ, Zarkower D: *Dmrt1*, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. Genes Dev 14:2587–2595 (2000).
Sánchez FJ, Vilain E: Genes and brain sex differences. Prog Brain Res 186:65–76 (2010).
Saunders PA, Perez J, Rahmoun M, Ronce O, Crochet PA, Veyrunes F: XY females do better than the XX in the African pygmy mouse, *Mus minutoides*. Evolution 68:2119–2127 (2014).
Sekido R, Lovell-Badge R: Sex determination and SRY: down to a wink and a nudge? Trends Genet 25:19–29 (2009).
Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, et al: A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240–244 (1990).
Soullier S, Hanni C, Catzeflis F, Berta P, Laudet V: Male sex determination in the spiny rat *Tokudaia osimensis* (Rodentia: Muridae) is not *Sry* dependent. Mamm Genome 9: 590–592 (1998).
Svingen T, Spiller CM, Kashimada K, Harley VR, Koopman P: Identification of suitable normalizing genes for quantitative real-time RT-PCR analysis of gene expression in fetal mouse gonads. Sex Dev 3:194–204 (2009).
Swain A, Zanaria E, Hacker A, Lovell BR, Camerino G: Mouse *Dax1* expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nature Genet 12:404–409 (1996).
Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, et al: Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139:1130–1142 (2009).
Vainio S, Heikkilä M, Kispert A, Chin N, McMahon AP: Female development in mammals is regulated by *Wnt-4* signalling. Nature 397: 405–409 (1999).
Veyrunes F, Chevret P, Catalan J, Castiglia R, Watson J, et al: A novel sex determination system in a close relative of the house mouse. Proc Biol Sci 277:1049–1056 (2010).
Veyrunes F, Perez J, Paintsil SN, Fichet-Calvet E, Britton-Davidian J: Insights into the evolutionary history of the X-linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked *Sry* gene. Sex Dev 7:244–252 (2013).
Wainwright EN, Wilhelm D: The game plan cellular and molecular mechanisms of mammalian testis development. Curr Top Dev Biol 90:231–262 (2010).
Warr N, Greenfield A: The molecular and cellular basis of gonadal sex reversal in mice and humans. Wiley Interdiscip Rev Dev Biol 1:559–577 (2012).
Wilhelm D, Washburn LL, Truong V, Fellous M, Eicher EM, Koopman P: Antagonism of the testis- and ovary-determining pathways during ovotestis development in mice. Mech Dev 126:324–336 (2009).

Manuscrit 5: “Masculinised Behaviour of XY females in a Mammal with Naturally Occuring Sex-Reversal”

Le dogme central de la différentiation sexuelle chez les mammifères a longtemps reposé sur le fait que la mise en place des caractères sexuels secondaires est exclusivement le fruit de l'action des hormones gonadiques. Cependant, ce point de vue a été mis à mal ces dix dernières années (Arnold 2012). Les gonades jouent bien sûr un rôle majeur dans les différences entre les sexes, mais il a été mis en évidence que des gènes portés par les chromosomes sexuels ont un impact direct sur le neuro-développement et la mise en place de comportements sexe-spécifiques (notamment l'agressivité et les soins parentaux ; revue dans Cox et al. 2014). Ces résultats ont été obtenus sur des souris de laboratoire dont le sexe est artificiellement inversé, et l'influence du complément en chromosomes sexuels sur le comportement n'a jamais été testée sur des individus dont l'inversion du sexe est naturelle. Deux raisons nous ont poussés à analyser le comportement des souris naines africaines : (i) le gène *Sry*, connu pour son influence sur la différentiation sexuelle du cerveau (Sekido 2014) est exprimé dans le cerveau des femelles X*Y (manuscrit 3) et (ii) les femelles X*Y ont un meilleur succès reproducteur que les femelles XX et XX* (manuscrit 1), qui pourrait en partie résulter de différences comportementales.

Pour faire le lien entre complément en chromosomes sexuels, comportement et succès reproducteur des femelles, différents traits comportementaux ayant une influence sur les interactions entre les mâles et les femelles ont été analysés : l'attractivité des trois types de femelles, leur agressivité et leur anxiété. Aucune préférence pour un type de femelle n'a pu être mise en évidence chez les mâles. Cependant, nous avons pu montrer que le complément en chromosomes sexuels avait un impact direct sur d'autres traits : (i) les femelles X*Y sont plus agressives que les femelles XX, les femelles XX* ayant un niveau d'agressivité intermédiaire ; (ii) les individus portant un chromosome Y (XY et X*Y) sont moins anxieux que les autres (XX et XX*). Les différences d'anxiété ont été confirmées par des dosages de corticostérone réalisés par nos soins, non présents dans le manuscrit. Ces résultats mettent en évidence un effet du chromosome Y sur l'anxiété, et un effet du Y et du X* sur l'agressivité. Le lien entre le comportement des souris naines et le meilleur succès reproducteur des femelles X*Y est discuté en détail dans le manuscrit, il en ressort que de telles différences comportementales pourraient avoir des répercussions importantes sur l'écologie de l'espèce.

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Masculinised Behaviour of XY Females in a Mammal with Naturally Occuring Sex Reversal

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Abstract

Most sex differences in phenotype are controlled by gonadal hormones, but recent work on laboratory strain mice that present discordant chromosomal and gonadal sex showed that sex chromosome complement can have a direct influence on the establishment of sex-specific behaviours, independently from gonads. In this study, we analyse the behaviour of a rodent with naturally occurring sex reversal : the African pygmy mouse *Mus minutoides*, in which all males are XY, while females are of three types : XX, XX* or X*Y (the asterisk designates a still unknown sex reversal mutation on the X). X*Y females show typical female anatomy and, interestingly, have greater breeding performances. We investigate the link between sex chromosome complement, behaviour and reproductive success in females by analysing several behavioural features that could potentially influence their fitness : female attractiveness, aggressiveness and anxiety.

Despite sex chromosome complement was not found to impact male mate preferences, it does influence some aspects of both aggressiveness and anxiety : X*Y females are more aggressive than the XX and XX*, and show lower anxiogenic response to novelty, like males. We discuss how these behavioural differences might impact the breeding performances of females, and how the sex chromosome complement could shape the differences observed.

With two copies of the X chromosome in females versus one X and a Y chromosome in males, male heterogamety is the norm in mammals. The X and the Y are very different both in size and gene content as the result of a long differentiation from an ancient autosomal pair (Marshall Graves, 2006). They also differ from autosomes in that they are enriched in genes involved in sexual differentiation and reproduction (Hurst and Randerson, 1999; Vallender and Lahn, 2004). Over the last decade, several studies have also highlighted the direct influence of sex chromosome genes on the establishment of sexually dimorphic behaviours (Cox et al., 2014), findings that contrast with the classical view that sex differences are due to the sole action of gonadal hormones during development (Arnold, 2012). The relative impact of sex chromosome complement versus gonadal sex on behaviour has been assessed using different transgenic laboratory mouse strains, such as the "Four Core Genotypes" model (FCG), in which a Sry-deleted Y chromosome and an autosomal Sry transgene produce XX and XY-Sry females (XXF and XYF) and XX+Sry and XY males (XXM and XYM) (De Vries et al., 2002). These studies reveal that while some sex differences in behaviour are influenced by gonadal sex, for instance chemo-investigation of bedding (XXM and XYM investigate more than XXF and XYF), others, such as certain aspects of aggressiveness, are influenced by sex chromosome complement (XYM and XYF are more aggressive than XXM and XXF) Gatewood et al. (2006). The study of FCG and other mouse models showed an influence of sex chromosomes on various other features : parental behaviour, sexual behaviour or social interactions (Gatewood et al., 2006; McPhie-Lalmansingh et al., 2008; Xu et al., 2012), but also on non-behavioural traits such as metabolism or brain function (Chen et al., 2013; Corre et al., 2014). The differences are independent from gonadal hormones and result from the action of some Y-linked genes (Sekido, 2014) and/or the number of X chromosomes (e.g. one copy for XYM vs. two copies for XXM) Bonthuis et al. (2012).

The influence of sex chromosomes on behaviour has been tested mostly on laboratory strain mice in which sex chromosome complement was genetically manipulated, but never in species in which an unusual mode of sex determination was shaped by natural selection. In mammals, there are indeed a few natural exceptions to the standard XX/XY sex determination system (SDS). For example, fertile XY females are found in several lemmings and South American grass mice species (Fredga and Bulmer, 1988) and both males and females are X0 in the Japanese spiny rat Tokudaia osimensis (Honda and Itoh, 1977) and the mole vole Ellobius lutescens (Just et al., 1995). Species with such unusual SDS are particularly relevant to further investigate the link between sex chromosome complement and behaviour.

The African pygmy mouse, Mus minutoides, a close relative of the house mouse, has recently been added to the short list of mammals with unusual SDS (Veyrunes et al., 2010). In populations from Southern up to Wes-

tern Africa, XY females are found amongst standard XY males and XX females (Veyrunes et al., 2013). Sex reversal (here meaning discordance between chromosomal and phenotypic sex) of these XY females is not linked to a mutation of the male sex determining gene Sry nor any other Y-linked gene, but rather to the X chromosome. Cytogenetics revealed that two different X chromosomes, varying in size and structure, segregate in these populations : the ancestral X and a rearranged one named X*. The latter bears a still unknown mutation preventing masculinization of X*Y embryos. So while all males are XY, there are three types of females with different sex chromosome complements : XX, XX* and X*Y (Veyrunes et al. 2010). The three types of females cannot be told apart phenotypically, they have a similar body mass and ano-genital distance, and all harbour typical ovarian structure (Rahmoun et al., 2014), which suggest similar levels of circulating hormones. However, their reproductive performances differ : unexpectedly, X*Y females produce significantly more offspring than the XX and XX* females despite the meiotic issues expected in heterogametic oocytes and the loss of unviable YY embryos. This advantage results from a greater litter size, a higher breeding probability when paired with a male and an earlier breeding onset (Saunders et al., 2014). The latter two features could relate to variation in female attractiveness, i.e. male preference for X*Y females, or other behavioural traits that could delay pair bonding with XX and XX* females.

In this study, we analyse several behavioural traits in the African pygmy mouse (female attractiveness, aggressiveness and anxiety in both sexes) in order to answer two questions : does sex chromosome complement affect behaviour independently from gonadal sex in a species with naturally occurring sex reversal and could behavioural differences account for the greater reproductive output of X*Y females.

METHODS

Animals

The fifty pygmy mice (13 males and 37 females) used for this study were kept and raised at the breeding facility (CECEMA) of the University of Montpellier, France. The origin of the founder animals, and housing conditions in the colony were described previously (Veyrunes et al. 2010 ; Saunders et al. 2014). For this study, at weaning, males and females were housed separately in cages : females were housed in same-sex groups of 3-4 individuals per cage and males set in individual cages (to prevent agonistic behaviours). They were provided with ad-libitum food and water, and light regime was set to 15 :9 h (light :dark). Females were genotyped by PCR amplification of the Y-specific Sry gene and/or non-invasive fibroblast cell-culture established from skin biopsy (Veyrunes et al. 2010).

Behavioural Tests

Experimental procedures were performed in accordance with European guidelines and with the approval of the French Ethical Committee on animal care and use (No. CEEA-LR- 12170). All animals went through the different tests in the following order : Two-way choice test (i.e. Y maze) to test male preferences, resident-intruder test to test female aggressiveness and light-dark box, and open field to evaluate anxiety in both sexes. Sample sizes are given in table 1. The tests were conducted between 1300h and 1900h with a minimum interval of a week between two tests. The pedigree of each animal was assessed and encounters between closely related animals were avoided. The average age of individuals at the beginning of the study was 264 +/- 56 days old (mean +/- s.d.). As the oestrous state of females is thought to influence different behavioural traits in rodents (Hyde and Sawyer, 1977; Bronson, 1979; Ho et al., 2001; Zinck and Lima, 2013), it was assessed before experiments using the "wet smear" method (Caligioni, 2009).

	Females			Males
	XX	XX*	X*Y	XY
Y Maze	12	11	12	13
Resident-Intruder paradigm	12	12	12	12
Light-Dark box	12	12	11	12
Open-Field	11	12	11	13

Table 1 : Number of mice involved in each behavioural test.

male mate choice Two-way male mate choice was performed using a Y maze as described by Smadja and Ganem (2002). Briefly, the apparatus consists of a transparent Y shaped maze, in which a male is introduced via the main branch (27cm, diam :4.5cm). At the end of the two other (secondary) branches (25cm, diam :4.5cm) are Plexiglas boxes (15x15x10cm) with two receptive (in oestrous) "stimulus" females of two different genotypes. Male-female interactions are limited by perforated doors separating the boxes from the secondary branches. Each male was tested three times : once with each of the three types of pairs (XX vs. XX*, XX vs. X*Y and XX* vs. X*Y). Each stimulus female was used twice, once against each other genotype. The order of presentation of the stimuli was randomized and no male encountered the same female twice. A test started as soon as a male entered the main branch and lasted for 10 minutes. To assess male preference, the time spent in each tube (exploring, in contact with the perforated door, and in interaction with the female through the holes of the door) was measured.

female aggressiveness The resident-intruder test was used to compare aggressiveness in the three types of females. this paradigm relies on the analysis of the aggressiveness of an individual in its territory (here a non-receptive "resident" female) towards an "intruder" (a male). Before the test, the female was isolated for at least a week, and then placed in a large (40x30x30cm) transparent box with

her own soiled bedding. 48 hours later, the male was introduced in the resident cage via a side door. Encounters lasted 10 minutes. Each male faced the three different types of females sequentially, with a minimum of seven days between two trials. The order in which males encountered the different female genotypes was randomized. The latency to first attack and the occurrence of agonistic behaviours (attacks and chases) directed by females were scored.

anxiety and locomotor activity Two tests were used to assess anxiety-related behaviours and motor activity of males and females. The "Light-dark box" design consists of two adjacent boxes (23*16*10 cm) separated by a small opening (6*6 cm). The light compartment is brightly lit from above and covered with a transparent lid, and the dark compartment is covered with a black lid. Mice were placed in the light box, and experiments lasted 10 minutes. We considered the first two minutes as a habituation period, and recorded the time spent in a static posture during that time to assess anxiogenic response to novelty. From minute two to minute 10, the time spent in the light compartment (classical measure of state anxiety in mice, Bourin et al. (2007)) and the distance covered in the whole device (to assess locomotor activity) were recorded. The "open field" is a round open area (diam :50 cm) with high walls, virtually divided in two areas : the central zone (20cm), and outer zone (20-50 cm). Mice were placed in the central zone, and tests lasted 10 minutes. In a similar way to the light-dark box, we recorded the time spent freezing during the first two minutes, then the time spent in the central zone (a common measure of anxiety, as anxious individuals are expected to stay on the periphery of the field, Prut and Belzung (2003)) and the total distance covered.

Data Acquisition and Analyses

The Y maze and resident intruder test were filmed and mice behaviours were recorded by one of us blindly and analysed with The Observer software (v 5.0.31, Noldus). For the light-dark box and open-field tests, up to four animals were tested simultaneously, and movements were tracked and recorded using an infra-red tracking device and video-tracking software (VideoTrack, v 3.10, Viewpoint).

Male mate preferences were assessed by pair comparisons of time spent in each secondary branch, in contact with each perforated doors and in interaction with each female through the holes, separately for each modality (XX vs. XX*, XX vs. X*Y and XX* vs. X*Y), using Wilcoxon signed rank tests. Correction for multiple testing was made using the Bonferroni procedure.

After controlling for normality of the response variables, multivariate analysis of variance (MANOVA) were performed to analyse our measures of anxiety (time spent in the light box/central zone), locomotor activity (distance covered) and anxiogenic response to novelty (logarithm of time spent immobile) in the light-dark box and open-field. The effect of presence vs. absence of sex chromosomes X* and Y and their interaction (that allows to discriminate the four genotypes) were assessed. Three covariates were used : the age of the individual at the time of the experiment, the order in which they were tested (12 groups of up to four animals, all animals were tested on the same day) and their position in the experimental device (upper/lower –left/right). Model simplifications were made using Likelihood ratio tests (LRT).

Concerning the resident intruder test, departures from normal distribution of response variables precluded the use of MANOVAs. Instead, we carried independent analyses, accounting for multiple testing by using the sequential Bonferroni correction (Holm, 1979). The effect of genotype on the latency to first attack and the number of aggressive bouts were analysed using generalized linear mixed model with respectively (i) an exponential distribution (that applies when the variable is the time to the first occurrence of an event, Fox (1993)) and (ii) a geometrical distribution (which provided best graphical fit to data). Male identity was added as a random variable, and female :male mass-ratio and trial number for the male (first, second or third) were added as fixed covariates. Model simplification was made using LRTs. All statistical analyses were performed using R (R team 2015).

RESULTS

No Male Preference for a Given Female Genotype

Results indicate that males spent the same amount of time in each side of the Y maze, in contact with the doors leading to the females, and interacting with the two females through the perforated doors, whichever set of females (XX *vs.* XX*, XX *vs.* X*Y or XX* *vs.* X*Y) they encountered in the maze (table 2).

Time spent in	XX *vs.* XX*		XX *vs.* X*Y		XX* *vs.* X*Y	
Secondary branches	204.6 +/- 73.9	177.2 +/- 69.1	167.6 +/- 66.8	170.6 +/- 81.7	191.9 +/- 91.8	163.9 +/- 96.9
	V=30, p=0.90		V=47, p=0.95		V=38, p=0.70	
Contact	82.6 +/- 36.4	92.5 +/- 51.0	80.3.6 +/- 47.2	95.2 +/- 54.9	95.2 +/- 54.9	68.6 +/- 53.9
	V=26, p=0.58		V=58, p=0.41		V=47, p=0.24	
Interaction	44.5 +/- 26.8	61.5 +/- 31.5	44.2 +/- 35.2	42.5 +/- 32.7	64.0 +/- 47.6	37.2 +/- 33.9
	V=20, p=0.28		V=51, p=0.74		V=56, p=0.041	

Table 2 : Results of the Two-way choice test. Total time spent (sec, mean +/- s.e.m.) by males in each side of the apparatus (secondary branches), in contact with the perforated doors, and in interaction with the female through the holes of the door. Statistics : Wilcoxon test. P-values are shown before Bonferonni correction.

*X*Y Females are More Aggressive than the Others*

Model simplifications are detailed in supplementary material S1. We found a significant effect (robust to sequential Bonferroni correction) of genotype on latency to first attack (χ^2_2=7.0, p=0.029) and number of attacks by the resident (χ^2_2=11.00, p=0.004) (figure 1). There was no effect of any the covariates on either trait (S1). Tukey HSD tests were used to test post hoc differences between the three pairs of genotypes. X*Y females were significantly faster to attack males than XX females (p=0.037) while XX* females were intermediate (XX vs. XX* : p=0.45, XX* vs. X*Y : p=0.27). The X*Y also attacked males more often than the XX and XX* (XX vs. X*Y, p=0.03 ; XX* vs. X*Y, p=0.01), the latter showing a similar level of aggressiveness (p=0.93).

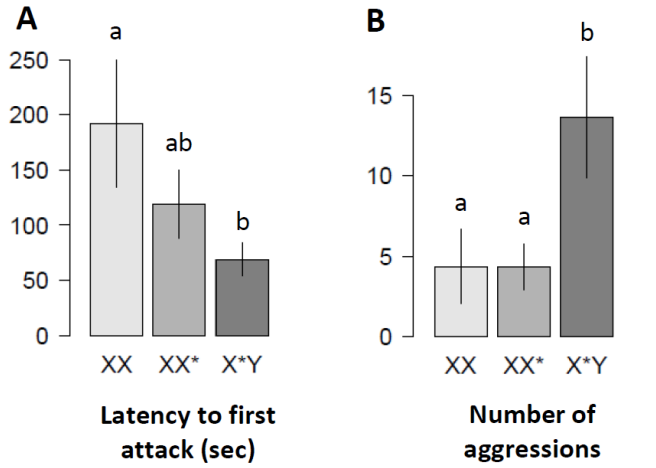

Figure 1 : Effect of female's genotype on Latency to first attack (A) and number of aggressions (B) by females in the Resident-intruder test (mean +/- s.e.m.). The letters above the bars indicate significant differences according to Tukey's HSD test.

Y Bearers are Less Anxious

Variables measured in the light-dark box and open-field are presented in figure 2. See S1 for detailed model simplification. The MANOVA performed on behavioural measures from the light-dark box indicates a significant effect of the Y chromosome (Pillai=0.20, $F_{1,41}$=3.34, p=0.03), but not of the X* (Pillai=0.02, $F_{1,38}$=0.28, p=0.66) nor of their interaction (Pillai :0.18, $F_{1,37}$=2.49, p=0.08) on behavioural variation. The age of mice was also found to affect their behaviour (Pillai=0.20, $F_{1,41}$=3.30, p=0.03). Univariate ANOVAs were performed to determine which response variables are affected by the presence vs. absence of the Y chromosome. Time spent in the light box was not affected ($F_{1,43}$=0.14, p=0.7), but mice carrying a Y chromosome (XY males and X*Y females) were found to spend less time immobile ($F_{1,41}$=10.97, p=0.002) during the habituation period, and covered a greater distance ($F_{1,43}$=4.64, p=0.04) after that period. Concerning the open-field, the MANOVA did not reveal any effect of the Y nor the X* (S1), despite similar trends observed on the time spent immobile and the distance covered (figure 2).

DISCUSSION

This study addresses the influence of sex chromosome complement on female behaviour and attractiveness to males in a mammal with unusual SDS : Mus minutoides. We found that X*Y females differ from XX and XX* females in respect to certain aspects of aggressiveness, anxiety and motor activity. In fact, sex-reversed females show behavioural features more similar to those of males, confirming the impact of sex chromosome complement on the behaviour of the African pygmy mouse.

Males and females face drastically different evolutionary pressures that are reflected in many sexually dimorphic traits (Darwin, 1871; Trivers, 1972). Such sex differences can be found in morphology (evolution of secondary sexual characteristics, like ornamentation or coloration), but also in the expression of behaviours (like aggressiveness or parental care, Kelley (1988)) . In M. minutoides, male-female behavioural dimorphism is reduced due to the presence of sex-reversed females that show masculinised behaviour.

The resident intruder test revealed that X*Y females show a shorter attack latency than XX females, (XX* females being intermediate ; figure 1A). They also attacked males more often than the females of the two other genotypes (figure 1B). In the light-dark box, sex-reversed females and males spent less time in a static posture than XX and XX* females at the beginning of the experiment, and a similar trend, yet non-significant, was observed in open field conditions (Figure 2C,F). This suggests a lower anxiogenic response to novelty in individuals harbouring a Y chromosome. However, we found no effect of the Y chromosome on the other classical parameters used to assess anxiety (time spent in brightly lit compartment in the light-dark box and in the centre of the open-field ; Figure 2A,D). This underlines the complexity of anxiety related behaviours, as shown by pharmacological studies : anxiety is not a unitary phenomenon, and different aspects of anxiety rely on different neurological and hormonal pathways (Lister, 1990; Belzung and Griebel, 2001). Finally, differences were also found in terms of motor activity : X*Y females and males show greater levels of motor activity than the XX and XX* females (Figure 2B,E). Overall, these findings are congruent with observations showing reduced behavioural dimorphism between sex-reversed females and males in laboratory strain mice (Gatewood et al., 2006; McPhie-Lalmansingh et al., 2008; Kopsida et al., 2013).

In a previous study (Saunders et al. 2014), we showed that female genotype has an influence on breeding success : X*Y females have a higher reproductive output thanks to a greater chance of having at least one litter, an earlier breeding onset (they have their first litter in average 20 days earlier than the XX and XX*) and the production of bigger litters. Some behavioural features (e.g. attractiveness) are known to impact fitness, so we hypothesised that the differences observed in terms of probability of breeding and age at first litter might result from behavioural diffe-

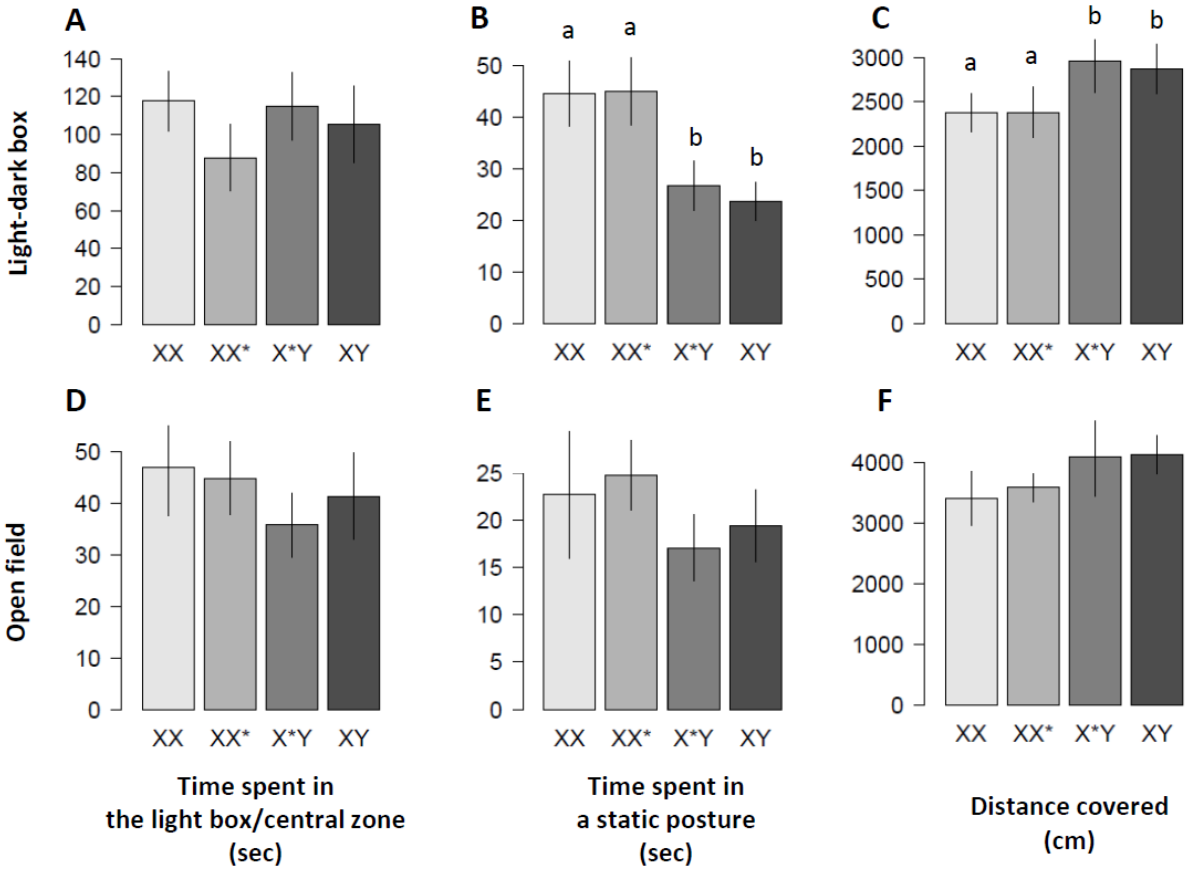

Figure 2 : Behavioural response of mice in the Light-dark box (A-C) and Open field (D-F) paradigms (mean +/- s.e.m.). Letters above the bars indicate a significant difference according to the univariate ANOVAs.

rences between female genotypes. Interestingly, the differences in behaviour highlighted in this paper follow the same pattern as in breeding success : XX and XX* differ from X*Y females. However, it is not straightforward how a reduced anxiety and an increased aggressiveness and locomotor activity might have a positive effect on fitness of X*Y females, especially as so little is known about the ecology of the African pygmy mouse (Britton-Davidian et al., 2012). The social and mating systems of this species have never been studied, which makes it hard to infer how these behavioural traits could impact breeding, but here are a few leads. Reduced anxiety of X*Y females could influence breeding success by facilitating male-female interactions. In female prairie voles, stress has been shown to inhibit pair bonding (DeVries et al., 1996), and in several other species, boldness is known to be positively correlated to fitness (Smith and Blumstein, 2007). The greater anxiety of XX and XX* females may explain why so many of these females do not breed in our colony, and why those that do have a delayed onset of reproduction. Aggression is also related to reproduction. If female pygmy mice are territorial (many female small mammals are, (Wolff, 1993)), the greater aggressiveness and locomotor activity of X*Y females could be advantageous to protect their offspring and provide adequate access to resources required for reproduction. These females could also attract more males and have more mates if their territories are bigger. Alternatively, if they live in social groups, aggressiveness could help achieving dominance and therefore a greater reproductive success. Also, in extreme cases, such as in Mus spicilegus, which belongs to the same genus as the African pygmy mouse, aggressiveness seems to be part of a "ritualised" sexual behaviour, triggering sexual motivation (Busquet et al., 2009). This greater aggressiveness might also be beneficial when considering the shift in sex-ratio caused by a feminizing mutation such as the X*. As some embryos with a Y become females rather than males, a female biased sex-ratio is expected in natural populations. This could alter the strength and direction of competition for mates, as mating becomes more difficult for the sex in the majority (Jiggins et al., 2000). Such conditions could favour the evolution of sex-role reversal : females would benefit from being more aggressive and less anxious while competing for males and choosiness might evolve in males.

Despite male preference for X*Y females may be advantageous, the experiments we conducted to test male preference (Y maze) did not reveal any male preference for one type of female over another (table 2). This does

not imply that choice is absent, as our experiments were restricted to short term olfactory and visual contact, and choice can be exerted in many ways (Edward and Chapman, 2011). Informal observations in our laboratory colony suggest that, in contrast with laboratory mice, pair formation could take several days/weeks (e.g. when they are first paired, it often takes several days before a male and a female can be found sharing the same nest, suggesting that it takes a certain time before they accept each other). As male preference could be a crucial feature in breeding performance (e.g. wild male house mice mated to preferred females have higher reproductive success, (Gowaty et al., 2003)), it should be studied more thoroughly. In addition, other behavioural experiments could be conducted, as it is not unlikely that differences in behaviour extend to other traits. For example, it has been shown using genetically manipulated laboratory mice that XY females tend to be more social than XX ones (McPhie-Lalmansingh et al., 2008; Cox and Rissman, 2011). So the study of social behaviour as well as sexual and parental behaviours (which have been found to be influenced by genes on the sex chromosomes (Gatewood et al., 2006; Grgurevic et al., 2012)) should help to clarify the link between sex chromosomes, behaviour and reproduction in M. minutoides.

Besides the evolutionary and ecological issues raised by these results, this study also supports recent findings concerning the direct effect of sex chromosomes on behaviour. During the last decade, there has been a growing interest in the "direct" role of the expression of sex chromosome genes on the shaping of sexual dimorphic behaviours (Cox et al. 2014), as opposed to the "indirect" way : trough the action of gonadal hormones (Kopsida and Stergiakouli, 2009). In the African pygmy mouse, the lack of noticeable differences between the anatomy and ovaries of XX, XX* and X*Y females (Rahmoun et al. 2014) could imply that all female have similar levels of circulating gonadal hormones (though this would have to be confirmed by hormonal assay). So the differences found in this study in terms of aggressiveness, anxiety and locomotor behaviour is likely to result from the direct influence of genes of the Y, X and X* chromosomes on the brain.

It is notoriously hard to assign behavioural modifications to naturally occurring genetic changes (Horton et al., 2014), but a few genes are known to have a direct effect on behaviour and would make good candidates to explain the behavioural differences found in the pygmy mouse. Sry and Sts, two genes harboured by the Y chromosome, have been shown to influence aggressiveness in mice (Guillot et al., 1995; Mortaud et al., 2010). These genes, and others of the non-recombining region of the Y, could be responsible for differences in anxiogenic response to novelty, locomotor activity and number of aggressions which dissociate Y chromosome bearers (X*Y and XY) from non-bearers (XX and XX*) in the pygmy mouse. Sry is a serious candidate, as it has been shown to be strongly expressed in the brain of X*Y females in the pygmy mouse (Rahmoun et al. 2014). In regard to attack latency, X*Y female differ from the XX, and XX* are intermediate, evoking an influence of the X*. More specifically, a gene that is expressed differently between the X and the X* could cause this pattern, as its level of expression would be intermediate in XX* females (due to random inactivation of the X). Monoamine oxidase A (MaoA) is an X-linked gene well known to influence behaviour : MaoA mice knockouts show increased aggressiveness (Cases et al., 1995). An X-X* difference in expression of MaoA could therefore explain the differences observed in terms of attack latency. Further genetic analyses (expression of candidate genes in the brain) as well as hormonal assays (pre and post-nataly) are required to disentangle the respective implication of direct and indirect effects of genes on the breeding performance of females in this species.

CONCLUSION

In this study, we show that sex chromosome complement has an impact on several behavioural traits in Mus minutoides, independently from gonadal sex : X*Y females show some masculinised behaviours despite their typical female anatomy. The African pygmy mouse is a promising model to further investigate the link between behaviour and sex chromosomes, especially since unlike other animal models used for this purpose, sex-reversal is a naturally occurring phenomenon in this species. It is also the first time a behavioural study has been conducted in a mammal with an unusual SDS. Females with either XX or XY sex chromosome complement can be found in a few other mammalian species. Examining behaviour in these species, as well as extending such studies to species with other types of unusual SDS in mammals, would help to better understand the ecological and evolutionary implications of the deviation from the standard XX/XY system.

References

Arnold, A., 2012. The end of gonad-centric sex determination in mammals. Trends Genet. 28 (2), 55–61.

Belzung, C., Griebel, G., 11 2001. Measuring normal and pathological anxiety-like behaviour in mice : a review. Behav. Brain. Res. 125 (1), 141–9.

Bonthuis, P. J., Cox, K. H., Rissman, E. F., 4 2012. X-chromosome dosage affects male sexual behavior. Horm. Behav. 61 (4), 565–72.

Bourin, M., Petit-Demoulière, B., Dhonnchadha, B. N., Hascöet, M., 12 2007. Animal models of anxiety in mice. Fund. Clin. Pharmacol. 21 (6), 567–74.

Britton-Davidian, J., Robinson, T., Veyrunes, F., 1 2012. Systematics and evolution of the african pygmy mice, subgenus nannomys : a review. Acta Oecol. 42, 41–49.

Bronson, F., 1979. The reproductive ecology of the house mouse. Q Rev. Biol. 54 (3), 265–99.

Busquet, N., Leveille Nizerolle, C., Feron, C., 1 2009. What triggers reproductive life ? effects of adolescent cohabitation, social novelty and aggression in a monogamous mouse. Ethology 115 (1), 87–95.

Caligioni, C., 2009. Assessing reproductive status/stages in mice. Curr. Protoc. Neurosci. 48 :4I :A.4I.

Cases, O., Seif, I., Grimsby, J., Gaspar, P., 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking maoa. Science 268 (5218), 1763–6.

Chen, X., McClusky, R., Itoh, Y., Reue, K., Arnold, A. P., 3 2013. X and y chromosome complement influence adiposity and metabolism in mice. Endocrinology 154 (3), 1092–104.

Corre, C., Friedel, M., Vousden, D. A., Metcalf, A., Spring, S., Qiu, L. R., Lerch, J. P., Palmert, M. R., 12 2014. Separate effects of sex hormones and sex chromosomes on brain structure and function revealed by high-resolution magnetic resonance imaging and spatial navigation assessment of the four core genotype mouse model. Brain Structure and Function, 1–20.

Cox, K. H., Bonthuis, P. J., Rissman, E. F., 1 2014. Mouse model systems to study sex chromosome genes and behavior : Relevance to humans. Front. Neuroendocrin. 35 (4), 405–19.

Cox, K. H., Rissman, E. F., 6 2011. Sex differences in juvenile mouse social behavior are influenced by sex chromosomes and social context. Genes Brain Behav. 10 (4), 465–72.

Darwin, C., 1871. The Descent of Man and Selection in Relation to Sex.

De Vries, G. J., Rissman, E. F., Simerly, R. B., Yang, L.-Y., Scordalakes, E. M., Auger, C. J., Swain, A., Lovell-Badge, R., Burgoyne, P. S., Arnold, A. P., 10 2002. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J. Neurosci. 22 (20), 9005–14.

DeVries, A., DeVries, M., Taymans, S., Carter, C., 1996. The effects of stress on social preferences are sexually dimorphic in prairie voles. PNAS 93, 11980–4.

Edward, D. A., Chapman, T., 12 2011. The evolution and significance of male mate choice. Trends ecol. evol. 26 (12), 647–54.

Fox, G. A., 1993. Failure-time analysis : emergence, flow- ering, survivorship, and other waiting times. In : Design and Analysis of Ecological Experiments. p. 253–89.

Fredga, K., Bulmer, M., 1988. Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with xy females. Phil. Trans. R. Soc. Lond. B 322 (1208), 83–95.

Gatewood, J. D., Wills, A., Shetty, S., Xu, J., Arnold, A. P., Burgoyne, P. S., Rissman, E. F., 2 2006. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. J. Neurosci. 26 (8), 2335–42.

Gowaty, P. A., Drickamer, L. C., Schmid-Holmes, S., 1 2003. Male house mice produce fewer offspring with lower viability and poorer performance when mated with females they do not prefer. Anim. Behav. 65 (1), 95–103.

Grgurevic, N., Büdefeld, T., Spanic, T., Tobet, S. A., Majdic, G., 5 2012. Evidence that sex chromosome genes affect sexual differentiation of female sexual behavior. Horm. Behav. 61 (5), 719–24.

Guillot, P.-V., Carlier, M., Maxson, S. C., Roubertoux, P. L., 7 1995. Intermale aggression tested in two procedures, using four inbred strains of mice and their reciprocal congenics : Y chromosomal implications. Behav. Genet. 25 (4), 357–60.

Ho, H. P., Olsson, M., Westberg, L., Melke, J., Eriksson, E., 5 2001. The serotonin reuptake inhibitor fluoxetine reduces sex steroid-related aggression in female rats : an animal model of premenstrual irritability ? Neuropsychopharmacol. 24 (5), 502–10.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scan. J. Stat. 6 (2), 65–70.

Honda, T., Itoh, M., 1977. An unusual sex chromosome constitution found in the amami spinous country-rat, tokudaia osimensis osimensis. Jpn J. Genet. 52 (3), 247–9.

Horton, B. M., Hudson, W. H., Ortlund, E. a., Shirk, S., Thomas, J. W., Young, E. R., Zinzow-Kramer, W. M., Maney, D. L., 1 2014. Estrogen receptor α polymorphism in a species with alternative behavioral phenotypes. PNAS 111 (4), 1443–8.

Hurst, L. D., Randerson, J. P., 10 1999. An exceptional chromosome. Trends Genet. 15 (10), 383–5.

Hyde, J., Sawyer, T., 1977. Estrous cycle fluctuations in aggressiveness of house mice. Horm. Behav. 295, 290–5.

Jiggins, F. M., Hurst, G. D. D., Majerus, M. E. N., 1 2000. Sex-ratio-distorting wolbachia causes sex-role reversal in its butterfly host. Proc. R. Soc. B 267 (1438), 69–73.

Just, W., Rau, W., Vogen, W., Akhverdian, M., Fredga, K., Marshall Graves, J. A. M., Lyapunova, E., 1995. Absence of sry in species of the vole ellobius. Nat. Genet. 11 (2), 117–18.

Kelley, D. B., 1988. Sexually dimorphic behaviors. Annu. Rev. Neurosci. 11, 225–51.

Kopsida, E., Lynn, P. M., Humby, T., Wilkinson, L. S., Davies, W., 1 2013. Dissociable effects of sry and sex chromosome complement on activity, feeding and anxiety-related behaviours in mice. PloS one 8 (8), e73699.

Kopsida, E., Stergiakouli, E., 2009. The role of the y chromosome in brain function. Open Neuroendocrinol J. 2, 20–30.

Lister, R. G., 1 1990. Ethologically-based animal models of anxiety disorders. Pharmac. Ther. 46 (3), 321–40.

Marshall Graves, J. A. M., 3 2006. Sex chromosome specialization and degeneration in mammals. Cell 124 (5), 901–14.

McPhie-Lalmansingh, A., Tejada, L., Weaver, J., Rissman, E., 2008. Sex chromosome complement affects social interactions in mice. Horm. Behav. 54 (4), 565–70.

Mortaud, S., Nicolas, L., Pinoteau, W., Tordjman, S., Carlier, M., Roubertoux, P. L., 3 2010. Brain pathways mediating the pro-aggressive effect of the steroid sulfatase (sts) gene. Behav. Genet. 40 (2), 211–9.

Prut, L., Belzung, C., 2 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors : a review. Eur. J. of Pharmacol. 463 (1), 3–33.

Rahmoun, M., Perez, J., Saunders, P. A., Boizet-Bonhoure, B., Wilhelm, D., Poulat, F., Veyrunes, F., 2014. Anatomical and molecular analyses of xy ovaries from the african pygmy mouse mus minutoides. Sex. Dev. 8 (6), 356–63.

Saunders, P. A., Perez, J., Rahmoun, M., Ronce, O., Crochet, P.-A., Veyrunes, F., 3 2014. Xy females do better than the xx in the african pygmy mouse, mus minutoides. Evolution 68 (7), 2119–27.

Sekido, R., 1 2014. The potential role of sry in epigenetic gene regulation during brain sexual differentiation in mammals. In : Epigenetic Shaping of Sociosexual Interactions : From Plants to Humans. Vol. 86C. Elsevier, pp. 135–65.

Smadja, C., Ganem, G., 2002. Subspecies recognition in the house mouse : a study of two populations from the border of a hybrid zone. Behav. Ecol. 13 (3), 312–20.

Smith, B. R., Blumstein, D. T., 12 2007. Fitness consequences of personality : a meta-analysis. Behav. Ecol. 19 (2), 448–55.

Trivers, R., 1972. Parental investment and sexual selection. In : Sexual Selection and the Descent of Man. B Campbell, pp. 136–79.

Vallender, E. J., Lahn, B. T., 2 2004. How mammalian sex chromosomes acquired their peculiar gene content. BioEssays 26 (2), 159–69.

Veyrunes, F., Chevret, P., Catalan, J., Castiglia, R., Watson, J., Dobigny, G., Robinson, T. J., Britton-Davidian, J., 4 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. B 277, 1049–56.

Veyrunes, F., Perez, J., Paintsil, S., Fichet-Calvet, E., Britton-Davidian, J., 2013. Insights into the evolutionary history of the x-linked sex reversal mutation in mus minutoides : clues from sequence analyses of the y-linked sry gene. Sex. Dev. 7 (5), 244–52.

Wolff, J., 1993. Why are female small mammals territorial ? Oikos 68, 364–370.

Xu, B., Obata, Y., Cao, F., Taketo, T., 1 2012. The presence of the y-chromosome, not the absence of the second x-chromosome, alters the mrna levels stored in the fully grown xy mouse oocyte. PLoS ONE 7 (7), e40481.

Zinck, L., Lima, S. Q., 1 2013. Mate choice in mus musculus is relative and dependent on the estrous state. PLoS ONE 8 (6), e66064.

Manuscrit 6: “Structural and sequence evolution of a third sex chromosome in the African pygmy mouse *Mus minutoides*”

Les chromosomes sexuels chez les mammifères ont un contenu en gènes très particulier, et portent notamment un excès de gènes impliqués dans la reproduction (revue dans Marshall Graves 2006). Comme expliqué dans l'introduction, cette spécialisation résulte de l'arrêt de la recombinaison et des patrons de transmission biaisés des régions non recombinantes. Chez *Mus minutoides*, avec l'apparition du chromosome X*, les patrons de transmission des chromosomes sexuels ont été modifiés : le chromosome Y a perdu sa transmission sexe-spécifique, le X passe plus de temps dans un contexte mâle que femelle, et la partie non-recombinante du X* a acquis une transmission limité à la lignée femelle. L'étendue de cette zone non recombinante est inconnue, mais des analyses cytogénétiques suggèrent qu'elle pourrait être large. Comment l'apparition de la mutation féminisante sur le X affecte-t-elle l'évolution des chromosomes sexuels chez la souris naine africaine?

Suite au séquençage complet du génome de la souris naine et un mapping sur le génome de référence de *Mus musculus*, la structure et la séquence des chromosomes X et X* ont été comparées, et plusieurs résultats suggèrent qu'ils ont commencé à se différencier. Plusieurs grandes zones diffèrent en terme de couverture entre les deux chromosomes (soit à cause de délétions ou d'amplifications). Certaines de ces régions avaient déjà été détectées grâce à une analyse d'hybridation génomique comparative (CGH-array) réalisée sur plusieurs autres individus, suggèrant que ces différences sont probablement fixées et donc dans des zones qui ont cessé de recombiner. Curieusement, les différences entre le X et le X* résultent majoritairement d'amplifications sur le X, alors que très peu de délétions ont été identifiées. Quelques gènes se trouvent dans ces régions, dont certains sont connus pour être impliqués dans la fonction mâle, pouvant laisser penser que le chromosome X est en train de se masculiniser. Ensuite, afin de déterminer l'étendue de la région non recombinante sur le X*, nous nous sommes attelés à essayer d'identifier des bornes d'inversions sur le X et le X* en se basant sur la comparaison des patrons de mapping des reads des deux chromosomes. Une dizaine de régions candidates ont été trouvées, et pourront être testées prochainement par PCR. Finalement, la divergence de séquence entre les deux chromosomes a été évaluée en analysant le polymorphisme nucléotidique (SNP). Il existe une forte hétérogénéité dans les patrons de divergence entre le X et le X* le long du chromosome, et de larges régions de divergence élevée, patron attendu dans les régions non recombinantes. Cette étude nous donne un premier aperçu de la divergence entre le X et le X* et montre que même si les différences ne sont pas énormes, les chromosomes ont commencé à se différencier.

Structural and sequence evolution of a third sex chromosome in the African pygmy mouse *Mus minutoides*

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INTRODUCTION

Among species that reproduce sexually, many harbor morphologically differentiated sex chromosomes. In mammals or drosophila, females possess two copies of the X chromosome (they are the homogametic sex) and males harbor one copy of the X and a Y chromosome (heterogametic sex). In other taxa such as birds, snakes or butterflies, female is the heterogametic sex (ZW), and male the homogametic sex (ZZ) (Bull 1983).

The emergence and evolution of sex chromosomes are attributed to the appearance of a sex determiner on a standard autosome – as first suggested a century ago by Muller (1914). In therian mammals for example, sex chromosomes are homologous to bird chromosomes 1 and 4 and gained their status around 150 Million years ago (Veyrunes *et al*. 2008), when a male sex determiner: *Sry*, was acquired by the proto-Y chromosome. At the time, except for this sex determining locus, the proto-X and Y were identical, which drastically contrasts with the current differences observed. Nowadays, in the vast majority of mammals, the Y chromosome is small, contains many repetitive sequences and lost most of its ancestral gene content (e.g. the mouse Y has retained only 9 out of 639 ancestral genes, Bellott et al., 2014). The remaining genes are all highly specialized in male reproductive functions (Bellott *et al*. 2014; Soh *et al*. 2014). The X chromosome on the other hand, is highly conserved and retained most ancestral genes (97% on the human X, Mueller et al. 2013). It nevertheless also shows a biased gene content, with an overrepresentation of genes involved in both male and female reproductive functions and genes highly expressed in the brain (reviewed by Marshall Graves, 2006).

The steps leading from undifferentiated proto-sex chromosomes to fully differentiated sex chromosomes have been extensively studied (reviewed in Bachtrog, 2006; Charlesworth, Charlesworth, & Marais, 2005; Marshall Graves, 2006). In brief, this process is initiated by the emergence of a sex determiner on one of the members of an autosomal pair, followed by a suppression of recombination, favored by the accumulation of sexually antagonistic genes in its vicinity. In the absence of recombination, sex chromosomes continue to differentiate, leading to the degeneration of the heterogametic sex chromosome and sexualisation of the gene content of both sex chromosomes[7].

In *Mus minutoides*, the way this complex set of evolutionary forces acts on the sex chromosomes must have been radically disturbed with the emergence around 1 MYA of a third sex chromosome, the feminizing X*, that harbors a female sex-determiner that overrides the action of *Sry* in X*Y embryos, preventing their differentiation into males. In this polygenic sex determination system, all males are XY, and females are either XX, XX* or X*Y (Veyrunes *et al*. 2010, 2013). The evolutionary trajectories of the Y, but also of the whole region in full sex linkage on the X and X* (i.e. the region that does not recombine between X and X*, the extent of which is still unknown) have probably been modified. The Y chromosome lost its male-specific transmission, reducing the advantage to male beneficial mutations conferred by constant selection. The effective size of the X (a shortcut to designate here the part of the X that does not recombine with X*) has been reduced, making it more vulnerable to drift, and the ratio of time it spends in male *vs*. female context has changed (it now spends more time in male context). Finally, the X* is never found in diploid conditions and could thus be prone to degeneration, and it acquired a female limited transmission that should favour its feminisation. These changes in the evolutionary forces acting could generate modifications in gene content (sexualisation), expression patterns (sex-biased), and sequence (rapid evolution under positive selection, or degeneration after suppression of recombination). The amplitude of the phenomenon would depend largely on the size of the non-recombining region, which is still unknown. However, we do have substantial evidence that the X and the X* have started diverging, based on several cytogenetics analyses. First, G-banding revealed the existence of structural differences between the X and the X* consistent across individuals: the X* is shorter than the X, and differs in terms of banding pattern (Veyrunes et al, 2010) suggesting the existence of

[7] The evolutionary forces responsible for shaping the peculiar gene content of the X and the Y in mammals are reviewed in table 1 of the introduction section.

chromosomal rearrangements. Preliminary results of BAC-mapping (Fluorescent *in-situ* hybridization) suggested the existence of inversions and/or translocations along these chromosomes[8]. Finally, array-CGH (Comparative Genomic Hybridization, a powerful method to scan entire genomes to detect DNA copy number variation (CNV, amplifications and deletions), Pinkel and Albertson 2005) revealed the existence of several regions with CNVs between the X and X* chromosomes[9].

To clarify the extent of the divergence between the X and X* and the impact of the new modes of transmission on these chromosomes, shotgun genome sequencing using next generation sequencing technologies (*Illumina HiSeq*) was performed for two *Mus minutoides* female specimens: one XX and one X*Y. After mapping on the house mouse reference genome, X and X* sequences were compared to try and answer the following questions: How big is the non-recombining region? How divergent (in structure and sequence) are the X-X* non recombining region(s)? What genes are affected by the rearrangements, and therefore might be candidates for sex-reversal? What are the functional consequences of divergence in the light of selection linked to the new modes of transmission (e.g.: degeneration/feminization of the X*)? Structural differences were assessed by analyzing regions with CNV and searching for inversion breakpoints (frequently observed in the early stages of sex-chromosome differentiation (Matsunaga 2006; Ross and Peichel 2008), and sequence divergence was studied by analyzing SNPs along the chromosome.

METHODS

DNA sampling, extraction and sequencing

DNA from two females (XX and X*Y) from our breeding colony was extracted from tail tips. The library preparations and genome sequencing were performed at the GATC-biotech company using the Illumina HiSeq2000 platform. One mate-pair library with an average targeted fragment size of 3Kb was constructed for each sample and 50 bp were sequenced at each extremity of these fragments. Four Hiseq2000 flowcell lanes were used to sequence the X*Y library, and two lanes for XX library. This imbalance aimed at

[8] See figure 4 in the introduction section
[9] See figure 5 in the introduction section

obtaining equivalent coverage for the X and X*, and resulted in 1878M read pairs for X*Y data, and 966M read pairs for XX data.

All the data processing and analyses were performed on the open source web-based platform *Galaxy* (using tools available on the main *Galaxy* instance and programs developed locally by the MBB (Montpellier Bioinformatics Biodiversity) platform), and *R*.

NGS data processing and mapping

First, the raw reads were quality and size filtered. Using *Trimmomatic (v0.32.1)*, adapters and other Illumina-specific sequences were removed from the reads. Then a sliding window trimming approach was performed by starting scan at the 5' end and clipping the 3' end of the read once the average sequencing quality within the window fell below a threshold set to 20 (a Phred quality score of 20 means that the probability of incorrect base call is of 1 in 100). Remaining adapters were removed using *Cutadapt* (*v0.8*), and reads smaller than 25bp were discarded.

Mapping on the *Mus musculus* (mm10) reference was performed using *NextGenMap* (*v0.4.5*), a fast and accurate read mapper with good performances when it comes to mapping reads highly divergent from the reference (Sedlazeck *et al*. 2013). We chose *NextGenMap* over the widely used *Bowtie 2* after making some performance comparisons based on a set of simulated read sets: we simulated five read sets (2x50bp mate pairs with a mean 3000bp insert size), derived from the *Mus musculus* mm10 reference, assuming an average divergence from the reference ranging from 2.5 to 12.5%. Simulated read pairs were mapped back to the mm10 reference using *Bowtie 2* with two types of settings (sensitive local mode with either 0 or 1 mismatch allowed in the seed) and *NextGenMap* with default parameters. Mapping percentage and true positive rates were estimated for the three mappings methods, and the results are shown in figure 1. The divergence between *Mus minutoides* and the reference being of around 7 million years (Veyrunes et al. 2005), we expect divergence to be high (roughly 6-10%). Our simulations show that *NextGenMap* gives better results than *Bowtie 2* in these conditions, especially considering how slow *Bowtie 2* (with 1 mismatch) is.

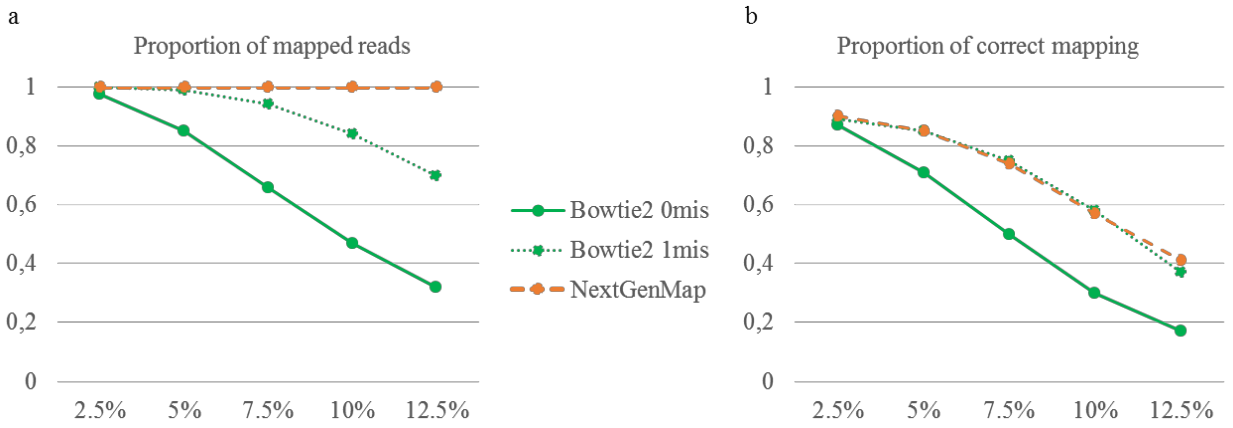


Figure 1: (a) proportion of simulated reads that mapped to the reference, depending on divergence with the reference (b) proportion of correctly mapped reads (amongst all reads).

Data from each Illumina lane were mapped separately, local realignments were made around INDELs, and PCR duplicates were removed using *rmdup* from *SAM Tools* (*v1.0.1*), that removes potential PCR duplicates by retaining only the pair with highest mapping quality if multiple read pairs have identical external coordinates. Files were filtered to keep only reads mapping on the reference X chromosome, with a decent mapping quality (MapQ≥20; error rate: 0.01). These two files, one containing all reads from the X chromosome, the other from the X*, were used for all further analyses.

Copy number variations

rSW-seq (recursive Smith-Waterman-seq) is a tool designed to identify CNVs between two genomes based on variations of sequencing depth along chromosomes (Kim *et al*. 2010). It is based on the Smith-Waterman algorithm, also used for analysis of array-CGH data. This method is highly sensitive and supposed to detect even a single copy change. In order to reduce the number of false positives we set up the threshold for *rSW-seq* significance level (the probability of finding the observed or more extreme distribution of reference and query reads in the identified region given the total number of genome1 and genome2 reads) to 1e-30 and to increase the threshold, the SW-score was set to 900. Besides that, we eliminated candidate CNVs that did not have coverage higher than three for one of the two samples.

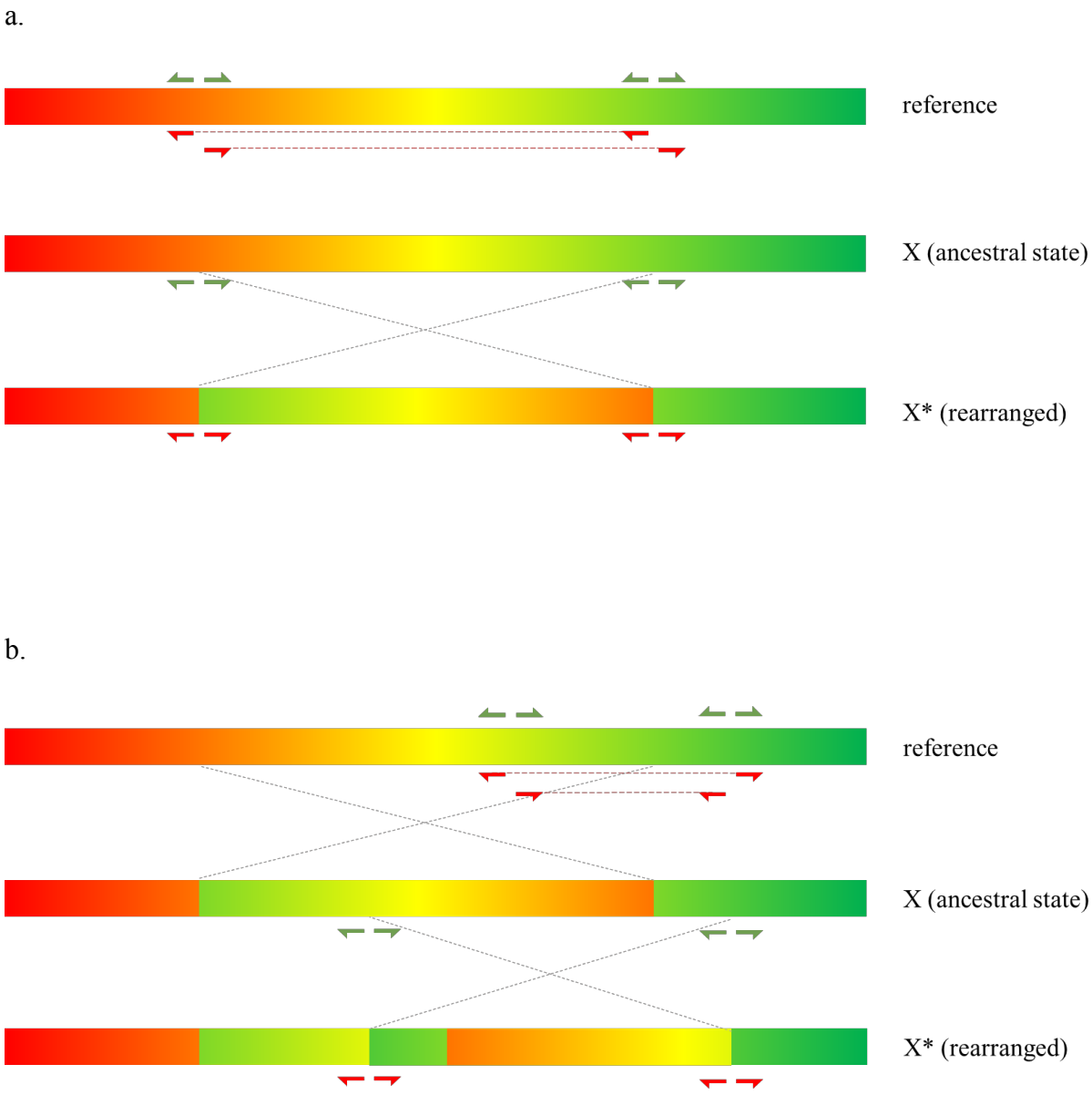


Figure 2. Comparison of the reference X chromosome, *Mus minutoides* X and X* chromosomes, assuming an inversion occurred on the X*, and expected mapping patterns around the inversion breakpoints. (a) Single inversion on the X*. (b) Overlap between an inversion preceding the divergence between the X and X* (found on both chromosomes) and a second inversion specific to the X*. The arrows (green for X and red for X*) represent read pairs flanking the breakpoints of the inversion of interest. The mapping patterns shown along the reference chromosome are those used to detect the inversion breakpoints. The colour gradient along the chromosome is just an aid to help visualising the rearrangements.

Inversions

An inversion can be detected by analyzing how read pairs map locally: read pairs flanking a breakpoint of an inversion between the genome they derive from and that used as a reference for mapping will map far apart and on the same strand on the reference (figure 2a). To identify inversions that distinguish the X and the X*, we searched for small regions (few kbs) containing a relative excess of reads that had mates mapping on the same strand (so called Forward – Forward or Reverse-Reverse configurations), and also clustered in a restricted region. We are here interested in genomic regions showing this pattern on one chromosome type (X or X*) but not the other, which is expected in case of a relative inversion between the two chromosomes. However, our preliminary BAC-mapping results suggest that the ancestor of the X and X* was already rearranged as compared to the *Mus musculus* X chromosome. If ever an inversion that distinguishes the X and the X* in *Mus minutoides* overlaps an inversion ancestral to their divergence, the mapping pattern will be different (leading to the Reverse-Forward and Forward-Reverse mapping configurations with abnormally high distance between pair mates for one of the genotypes, figure 2b). So we also searched for groups of pairs which mates "face" each other, and map far apart.

These patterns were searched for in non-overlapping 1kb regions. Only regions in which at least 30 reads mapped (for each sample) were analysed. The fraction of reads conforming to the first pattern (mate maps on same strand), and the second (mate faces the read, and insert size is superior to mean insert size) were assessed for X and X* data. All regions with a minimum 15% difference in the percentage of either type of reads between the two data set were kept. Finally, regions were considered as good candidates if these reads' mates mapped in a restricted region, a property that we assessed by analyzing the standard deviation of their position.

Sequence divergence

SNPs were called using *mpileupN* (*Samtools*). Only sites with depth of coverage over 5 were kept for further analyses. We kept at each position the most common allele, provided that it was observed at least 5 times (i.e. we did not try to resolve heterozygosity in the XX individual). Only positions that varied in our dataset (X, X* and the reference) were kept for further analyses. In sliding windows of 1000 adjacent SNP positions we calculated the distance between X and X* (number of positions where they differ) standardized by their average distance to the reference.

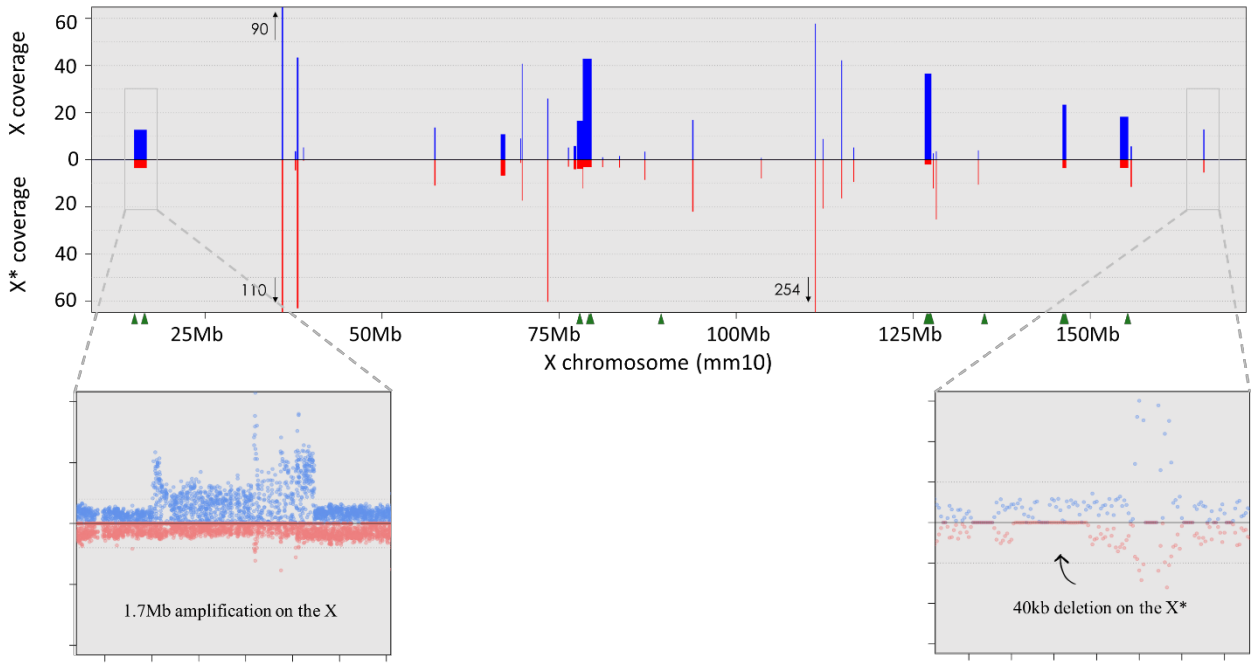


Figure 3. The top graph shows zones detected as CNV regions by rSW-seq analysis along the X chromosome. In each zone is shown the average depth of coverage for the X *vs*. X* (blue / red). Green arrows at the bottom of the graph indicate the location of CNVs detected with aCGH. The two bottom plots present coverage data of two of these regions, calculated from window size of 1kb using *SAM tools*, the first shows a large amplification on the X, and the second a 40kb deletion on the X*.

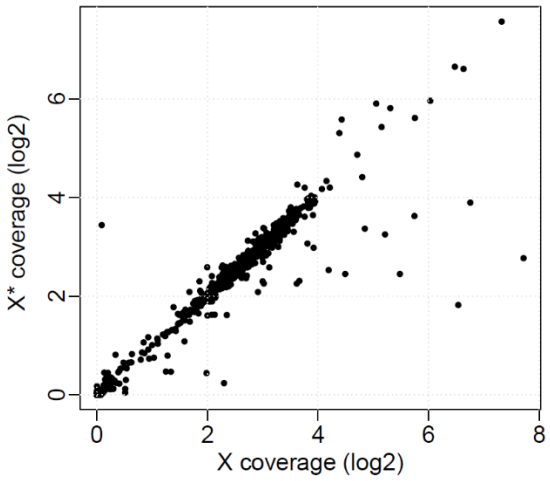


Figure 4. Coverage of the 936 protein coding genes on the reference X chromosome. Scale is $\log_2 +1$ (it is not unusual to add a pseudo-count of one to all counts so that counts withs zero coverage return zeros after log transformation).

RESULTS

CNVs

A total of 31 regions were detected as CNV regions by rSW-seq (figure 3). Eight out of nine of the zones identified by aCGH were also found here. Most CNVs consist of amplifications on one of the *Mus minutoides* chromosomes, most often on the X. Very few deletions were found on either chromosome, the biggest being a 40kb deletion on the X* (shown in fig 3).

To identify precisely the genes affected by these CNVs, we inspected the coverage data in 1kb sliding windows in the vicinity of the CNV regions pinpointed by rSW-seq. We found some discrepancies between the boundaries delivered by rSW-seq and those that could be inferred by visual inspection with the sliding window approach.[10].

We also calculated the mean coverage for each protein coding gene from the X and X* datasets. There was a very good correlation of the coverages between X and X*, indicating that overall coverage is a good predictor of copy number in this experiment (figure 4). The coverage fold change of each gene was measured. We limited ourselves to a descriptive analysis because of the lack of biological replicates normally necessary for significance analyses of that kind of data. We kept the 30 genes with the greatest coverage difference as genes potentially amplified or deleted on either chromosome ($\log_2$ fold change>0.75, table S1). Most of the genes are more covered on the X than on the X* (24/30), and the majority seem amplified on the X, but not on the X*, as compared to the reference. Among this list, 11 genes are known to be involved in reproductive functions or have a sexually dimorphic action. Most of the others are predicted genes with unknown functions (*GMs* and *Riks*).

[10] a couple of things still need to be clarified concerning the use of rSW-seq, that is also why I did not provide a list of CNV regions with start and end points

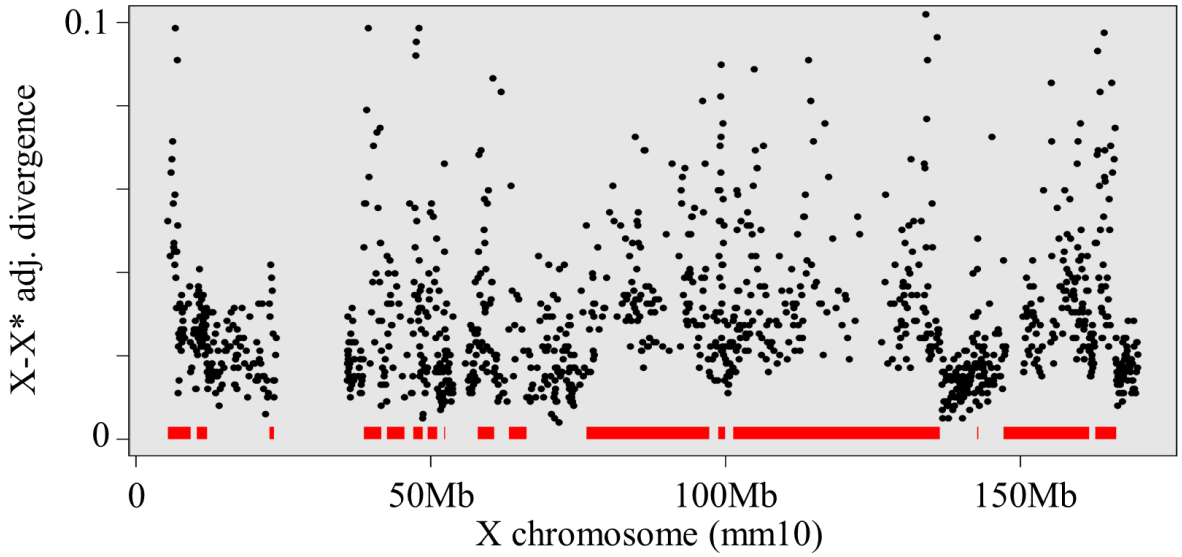


Figure 5. X-X* divergence along the chromosome. Divergence is adjusted by standardizing by the average distance to the reference ($\frac{nb\ SNPs\ X \neq X^*}{nb\ \ SNPs\ X=X^* + {}^1/_2 nb\ SNPs\ X \neq X^*}$, where "$nb\ SNPs\ X \neq X^*$" is the number of SNPs that distinguish the X and X* and "$nb\ SNPs\ X = X^*$", the number of SNPs that X and X* have in commun). Each dot represent divergence in a zone of 1000 SNPs. The red line represents regions of relatively high divergence, as given by a Hidden Markov Model.

Inversions

Twelve regions (table S2) contain reads that support the existence of simple inversions (figure 2a). The size of these putative inversions range from just above 1kb to over 400kb, most of them being rather small (a couple of kbs). Interestingly, one suspected inversion is supported by reads in two adjacent regions (reads spanning from 156.166 to 156.168 Mb) on the X* chromosome. Half of the presumed inversion breakpoints were found on the X and the other half on the X*.

Six candidate regions (table S2) were found for potential zones containing breakpoints of inversions following the second pattern investigated (figure 2b) and five out of six regions are harbored by the X chromosome.

Overall, it seems that these candidate breakpoints are concentrated mostly at the middle of the chromosome (77-116Mb), and to a lesser extent near the two ends of the chromosomes (15-16Mb and 156-163Mb).

Sequence divergence

There was a total of 29 769 487 sites covered by both data sets (X and X*) with a minimum depth of coverage of 5. (X mean coverage: 11.62, X* mean coverage: 10.78). Out of these, there were 1 225 311 sites (4.12%) for which X and X* were identical but different from the reference, and 35 684 sites (0.14%) at which X and X* differed (and thus one of them was like the reference since we considered only two state SNPs). The mean divergence (standardised by their average distance to the reference) between the X and X* was of 2.87%, variations in divergence along the chromosome are presented on figure 5, where it can be seen that it spans a wide range of values, with a tendency for windows of low and high divergence to cluster. A Hidden Markov Model (HMM, tool designed to represent probability distributions over sequences of observations, using a discrete variable, Rabiner and Juang 1986), set to categorize regions into high and low divergence zones, showed that there are large regions with consistent level of divergence, in particular two such large regions in the center of the chromosome. Note that the order of the sequences is that of the reference, so some disjoint blocks of high divergence pinpointed here could in fact be contiguous on the *minutoides* chromosome.

DISCUSSION

No signs of degeneration on the X*, amplifications on the X

Results given by rSW-seq are concordant with the aCGH results (eight out of nine regions identified by aCGH were detected here). Three biological replicates were used for aCGH, and the same CNV regions were detected each time. This suggests that these differences between the X and the X* might be fixed, and that these regions might be comprised in zones that stopped recombining. The extra regions detected with rSW-seq might be too small to be detected with aCGH, or just be unfixed polymorphisms in regions still recombining between the X and X*. They might also be regions where the probes of the CGH array did not hybridize properly.

Compared to CNV detection based on cytogenetics, the sequencing approach provides valuable additional information on the nature of the CNVs, by looking at coverage in the regions adjacent to the CNV, we can stipulate whether higher coverage in one sample is linked to DNA amplification or a deletion in the other sample (assuming the ancestral state is the absence of CNV). Most regions show a higher coverage on the X (Figure 3) and quite surprisingly, the inspection of X *vs*. X* coverage revealed that most CNVs are due to amplifications on the X rather to deletions on the X*. Several protein coding genes were found to be differentially covered on the two chromosomes. Some of them fall in the regions detected both by aCGH and rSW-seq (Maoa, Maob, Acot9, Prxd4…), and most of them are amplified on the X (figure 4, table 1).

These results were a little counter-intuitive for us. Indeed, despite a lack of evidence, the differences in coverage previously detected with aCGH had been assumed to result from deletions on the X*, due to the fact that degeneration of heterogametic chromosomes has long been considered like an ultimate fate for these non-recombining chromosomes (Ohno 1967). It's the case in mammals for example, in which the evolution of sex chromosomes was punctuated by several recombination arrests resulting in the formation of several evolutionary strata (Lahn and Page 1999). Each time a new region lost recombination, it underwent a rapid (negative exponential) decay (Bellott et al. 2014).

If very few deletions are found on the X*, does it mean it is still fully recombining with the X in XX* females? Not necessarily. The selective pressures to prevent decay must be much stronger on the X* compared to a standard Y or W chromosome. Indeed, in a

standard XX/XY sex determination system, the deleterious effects of gene loss on the Y chromosome are limited by the presence of homologous genes on the X, and can be balanced by dosage compensation (e.g. higher expression of genes on the X in a XY context). In contrast, the genes on the X* have no homologues in X*Y females, so a genetic decay would be extremely detrimental to these females.

A rapid search for functions of the affected genes revealed that some of them are involved in reproductive functions, sex specific processes or behaviour. Several are known to be involved in spermatogenesis and sperm function (Samt2, Prdx4, and Sms), and their amplification on the X can be viewed as the result of positive selection in males following the modification of the time the X spends in males relatively to females, leading to masculinization of this chromosome. Alternatively, amplification of genes expressed during spermatogenesis could be due to genomic conflicts between the sex chromosomes. Indeed, segregation distortion is a major force driving gene amplification on the mouse X and Y chromosomes (Soh et al. 2014), and for example, an evolutionary arms race between segregation distorters and repressors is thought to have driven the amplification of post-meiotically expressed testis genes such as *Slx* and *Sly* (Ellis *et al*. 2011; Cocquet *et al*. 2012). Overall, sexualisation of gene content (masculinization of the X and feminization of the X*) could be confirmed using expression analyses based RNAseq data.

Several candidate breakpoints for inversions were detected

Almost 20 candidate regions were identified as containing potential inversion breakpoints, either on the X or the X* (table 2), and will merit closer examination. Most candidate inversions are small, ranging from a couple of kb to a couple of Mb, and none is as big as what we could have expected based on the hybridization of mouse BACs on the X and X* (using Fluorescent *In-Situ* Hybridization, preliminary work). However, as argued in the Methods section, a large inversion could be hidden due to the divergence with the reference. For example, the overlap of two inversions, one preceding and one following the divergence of the X and X*, would not necessarily be detected as big as it really is using genomic data mapped on the house mouse genome (e.g. figure 2b). It could be argued that large inversions on the X are unlikely, as the X chromosome is highly conserved across mammals (e.g. Delgado et al. 2009). However the X chromosomes of murid rodents are surprisingly variable, and have not been exempt of internal rearrangements (Kuroiwa et al.

2001; Chiwalla et al. 2002), so an inversion between the reference X and ancestral *minutoides* is far from unlikely.

These results demonstrate the limits of analyzing genome rearrangements based on the mapping of reads on such a distant reference. These analyses would be greatly facilitated if the X chromosome of *Mus minutoides* was *de-novo* assembled, which requires a higher coverage and is greatly facilitated by multiple insert-size libraries, which we don't have for the moment.

The hypothetical breakpoints could be tested using PCR, by designing specific primers that bind upstream and downstream of these candidates. Noteworthy, if a diagnostic inversion is found between the X and X*, female karyotyping, which currently necessitates laborious cytogenetic techniques to distinguish XX and XX* females, could be greatly facilitated. Three specific primers could be designed around the breakpoint, one outside the inversion and two inside, one pair (inside + outside) would amplify a fragment on the X, and the other pair a fragment on the X*. If the two fragments amplified vary in size, a single PCR would be enough to distinguish the three types of females: on band for XX and X*Y females (of different sizes) and two bands for XX* females.

Sequence divergence

When comparing two chromosomes that have stopped recombining, higher divergence values correspond to greater times since recombination suppression. Here, there is a strong heterogeneity in X-X* sequence divergence along the chromosomes, with large zones of homogeneous elevated divergence (e.g. between around 69 and 135Mb, figure 5). We can speculate that the zones of elevated X-X* divergence might be the regions that have stopped recombining. The inversion breakpoints would not lie at the edge of these regions but rather somewhere in their center as recombination would be reduced on both sides of the breakpoints. The sharp transitions between high and low divergence regions that can be seen on figure 5 are most likely artefacts due to inversions anterior to the emergence of the X* (rearrangements between the reference and both the X and X*). Once again, our understanding of structural variations between the X and X* are limited by the important sequence divergence with the reference. However, the identification of diagnostic inversion breakpoints, either based on genomics or cytogenetic data will greatly help explaining these patterns.

CONCLUSION

The comparison of the structure and sequence of the X and X* sex chromosomes in *Mus minutoides* based on NGS data gives results in line with previous cytogenetic studies. They are concordant with the existence of a loss of recombination over a large fraction of the chromosomes. These results are very preliminary, but did reveal a couple interesting features of the chromosomes, in particular the existence of many amplifications on the X chromosome. These amplified regions comprise several genes, many of which are known to be highly expressed in testis, suggesting that the X chromosome is getting masculinized. This is in agreement with the fact that the evolutionary trajectories of sex chromosomes have been altered with the emergence of the X*, and that the X chromosome now spends more time in a male context. On the other hand, we could have expected to find signs of degeneration on the X* (deletions), because of its uniparental transmission, but surprisingly it was not the case. Sexualisation of X and X* chromosomes could be analysed more thoroughly using expression analyses based on RNAseq data. More effort also needs to be put in the identification of the non-recombining region, as well as the studying of the patterns of divergence in this region, as it will likely narrow down the list of candidate genes that might be responsible for sex-reversal.

This model is promising to better understand the impact of variation in selection regimes (e.g. underpinned by the modification of the modes of transmission) on the evolution of sex chromosomes. To go further, a *de-novo* assembly of the X chromosome of the African pygmy mouse is required and will greatly facilitate the comparison of the two chromosomes.

PERSPECTIVES

A serious drawback of this work is the high divergence – both in structure and sequence – between *Mus minutoides* X chromosomes and the mouse reference X chromosome. An important part of our data was lost because reads weren't properly mapped (if not at all), and the rearrangement ancestral to the divergence of the X and the X* makes the interpretation of sequence divergence tricky.

To get around these problems, we intend to improve mapping by updating the reference with SNPs detected on the X chromosome of *Mus minutoides* using several rounds of iterative mapping. This should guarantee a better depth of coverage for our two datasets. Also, Diethard Tautz provided us with sequencing data from *Mus mattheyi*, that was also mapped to the house mouse reference using NextGenMap (Neme and Tautz 2015). *Mus mattheyi* being closer to *Mus mintoides* (3.2 Myr ; Veyrunes et al. 2005), it will provide a better outgroup for the divergence analysis. Finally, to clarify this analysis, we will concentrate on gene divergence by measuring the ratio of the number of nonsynonymous substitutions per non-synonymous sites (d_N/d_S) of all genes with sufficient coverage all the chromosome.

REFERENCES

Bachtrog, D. 2006. A dynamic view of sex chromosome evolution. Curr. Opin. Genet. Dev. 16:578–585.

Bellott, D. W., J. F. Hughes, H. Skaletsky, L. G. Brown, T. Pyntikova, T.-J. Cho, N. Koutseva, S. Zaghlul, T. Graves, S. Rock, C. Kremitzki, R. S. Fulton, S. Dugan, Y. Ding, D. Morton, Z. Khan, L. Lewis, C. Buhay, Q. Wang, J. Watt, M. Holder, S. Lee, L. Nazareth, J. Alföldi, S. Rozen, D. M. Muzny, W. C. Warren, R. a Gibbs, R. K. Wilson, and D. C. Page. 2014. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. Nature 508:494–9.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park. CA.

Charlesworth, D., B. Charlesworth, and G. Marais. 2005. Steps in the evolution of heteromorphic sex chromosomes. Heredity (Edinb). 95:118–128.

Chiwalla, A. T., L. Cook, and Members of the Mouse Genome Analyysis Group for Mouse Genome Sequencing Consortium. 2002. Initial sequencing and comparative analysis of the mouse genome. Nature 420:520–62.

Cocquet, J., P. J. I. Ellis, S. K. Mahadevaiah, N. a Affara, D. Vaiman, and P. S. Burgoyne. 2012. A genetic basis for a postmeiotic x versus y chromosome intragenomic conflict in the mouse. PLoS Genet. 8:e1002900.

Delgado, C. L. R., P. D. Waters, C. Gilbert, T. J. Robinson, and J. a M. Graves. 2009. Physical mapping of the elephant X chromosome: conservation of gene order over 105 million years. Chromosome Res. 17:917–26.

Ellis, P. J. I., J. Bacon, and N. a Affara. 2011. Association of Sly with sex-linked gene amplification during mouse evolution: a side effect of genomic conflict in spermatids? Hum. Mol. Genet. 20:3010–21.

Kim, T.-M., L. J. Luquette, R. Xi, and P. J. Park. 2010. rSW-seq: algorithm for detection of copy number alterations in deep sequencing data. BMC Bioinformatics 11:432.

Kuroiwa, A., K. Tsuchiya, T. Watanabe, H. Hishigaki, E. I. Takahashi, T. Namikawa, and Y. Matsuda. 2001. Conservation of the rat X chromosome gene order in rodent species. Chromosom. Res. 9:61–67.

Lahn, B. T., and D. C. Page. 1999. Four Evolutionary Strata on the Human X Chromosome. Science (80-.). 286:964–967.

Marshall Graves, J. A. M. 2006. Sex chromosome specialization and degeneration in mammals. Cell 124:901–14.

Matsunaga, S. 2006. Sex chromosome-linked genes in plants. Genes Genet. Syst. 81:219–226.

Mueller, J. L., H. Skaletsky, L. G. Brown, S. Zaghlul, S. Rock, T. Graves, K. Auger, W. C. Warren, R. K. Wilson, and D. C. Page. 2013. Independent specialization of the human and mouse X chromosomes for the male germ line. Nat. Genet. 45:1083–7. Nature Publishing Group.

Muller, H. 1914. A factor for the fourth chromosome of Drosophila. Science (80-.). 39.

Neme, R., and D. Tautz. 2015. Entire genome transcription across evolutionary time exposes non-coding DNA to de novo gene emergence. BioRxiv, doi: 10.1101/017152.

Ohno, S. 1967. Sex Chromosomes and Sex-linked Genes. Springer-Verlag.

Pinkel, D., and D. G. Albertson. 2005. Array comparative genomic hybridization and its applications in cancer. Nat. Genet. 37 Suppl:S11–7.

Rabiner, L., and B. Juang. 1986. An introduction to hidden Markov models. ASSP Mag. IEEE.

Ross, J. a, and C. L. Peichel. 2008. Molecular cytogenetic evidence of rearrangements on the Y chromosome of the threespine stickleback fish. Genetics 179:2173–82.

Sedlazeck, F. J., P. Rescheneder, and A. von Haeseler. 2013. NextGenMap: fast and accurate read mapping in highly polymorphic genomes. Bioinformatics 29:2790–1.

Soh, Y. Q. S., J. Alföldi, T. Pyntikova, L. G. Brown, and T. Graves. 2014. Sequencing the Mouse Y Chromosome Reveals Convergent Gene Acquisition and Amplification on Both Sex Chromosomes. Cell.

Veyrunes, F., J. Britton-Davidian, T. J. Robinson, E. Calvet, C. Denys, and P. Chevret. 2005. Molecular phylogeny of the African pygmy mice, subgenus Nannomys (Rodentia, Murinae, Mus): implications for chromosomal evolution. Mol. Phylogenet. Evol. 36:358–69.

Veyrunes, F., P. Chevret, J. Catalan, R. Castiglia, J. Watson, G. Dobigny, T. J. Robinson, and J. Britton-Davidian. 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. B 277:1049–56.

Veyrunes, F., J. Perez, S. N. C. Paintsil, E. Fichet-Calvet, and J. Britton-Davidian. 2013. Insights into the evolutionary history of the X-Linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked Sry gene. Sex. Dev. 7:244–52.

Veyrunes, F., P. D. Waters, P. Miethke, W. Rens, D. McMillan, A. E. Alsop, F. Grützner, J. E. Deakin, C. M. Whittington, K. Schatzkamer, C. L. Kremitzki, T. Graves, M. a Ferguson-Smith, W. Warren, and J. a Marshall Graves. 2008. Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. Genome Res. 18:965–73.

SUPPLEMENTARY MATERIAL

gene	start	end	X coverage	X* coverage	Log_2 fold change
Maoa	16619698	16687818	21,50	4,45	2,21
Maob	16709282	16817366	6,99	3,92	0,80
Btg1-ps2	38038199	38043114	32,20	58,76	-0,86
Rhox10	38066475	38071691	19,91	38,49	-0,94
Gm6760	64151405	64152198	4,10	2,06	0,91
Zfp92	73411096	73428385	20,54	46,81	-1,18
Tbl1x	77511013	77662983	13,02	7,37	0,80
Gm14744	77864758	77870033	3,93	0,18	3,29
5430402E10Rik	77919786	77925062	0,42	0,03	1,27
4930480E11Rik	78369643	78371128	0,06	9,85	-5,01
Gm7173	79266559	79517285	91,88	2,53	5,05
Mageb4	86250254	86305093	1,43	0,73	0,77
Pet2	89403848	89409689	27,79	9,31	1,55
Gm44	90892142	90893134	2,95	0,35	2,41
AU015836	93968659	93975470	14,15	6,88	1,01
Taf1	101532734	101601789	6,54	3,23	0,96
1700011M02Rik	102908905	102909651	3,03	8,94	-0,97
Zcchc13	103630586	103631670	11,25	3,76	1,52
Ube2dnl1	114905012	114905941	17,28	4,76	1,81
Ube2dnl2	114907582	114908510	36,09	8,51	2,05
Tgif2lx2	118427227	118428256	1,38	0,38	1,36
Gm382	127039972	127063986	209,43	5,81	5,11
4921511C20Rik	127394293	127395898	43,64	4,46	3,22
Tmsb15b2	136955265	136958025	0,10	0,36	-0,82
Esx1	137115397	137122083	11,66	3,95	1,51
4930524N10Rik	154339225	154343392	7,10	2,79	1,50
Samt2	154575228	154579360	0,43	0,09	1,01
Acot9	155262443	155297654	107,01	13,87	2,93
Prdx4	155323918	155340754	52,58	11,32	2,19
Sms	157443855	157492287	7,11	3,77	0,87

Table S1. Protein coding genes on the X with the highest log_2 coverage fold change (>0.75). Genes in bold are genes with a known function related to reproduction, according to NCBI general gene information. A positive log_2 fold change means the X is more covered, a negative one means the X* is more covered.

Region		Read count		Same strand reads		Insert size (mean +/- sd)
Start (bp)	**End** (bp)	X	X*	X	X*	
15 679 001	15 680 000	300	76	**0,39**	0,13	2073,30 +/- 567,67
22 944 001	22 945 000	45	51	0,09	**0,47**	2935,39 +/- 330,03
86 047 001	86 048 000	115	164	**0,25**	0,02	1746,10 +/- 389,47
92 890 001	92 891 000	30	33	0,00	**0,33**	25826,38 +/- 319,17
100 270 001	100 271 000	120	81	**0,28**	0,02	19262,76 +/- 442,36
103 511 001	103 512 000	252	71	**0,20**	0,00	22056,38 +/- 242,63
106 661 001	106 662 000	47	31	**0,28**	0,03	2502,83 +/- 478,69
116 613 001	116 614 000	40	35	0,68	**0,89**	1579,57 +/- 638,81
156 166 001	156 167 000	36	32	0,00	**0,53**	406002,14 +/- 222,13
156 167 001	156 168 000	57	45	0,00	**0,62**	407830,92 +/- 463,93
156 493 001	156 494 000	59	72	**0,27**	0,04	5462,32 +/- 1054,79
163 964 001	163 964 001	44	52	0,02	**0,29**	3346,88 +/- 2834,15

Region	Region	Read count		Reads facing each other		Insert size (mean +/- sd)
Start (bp)	Start **(bp)**	X	X*	X	X*	
15 010 001	15 011 000	250	64	**0,332**	0,000	1720597 +/- 144,9
16 731 001	16 732 000	383	83	**0,272**	0,000	1720542 +/- 146,9
77 747 001	77 748 000	164	73	**0,195**	0,014	211101,4 +/- 390,9
91 523 001	91 524 000	35	42	**0,200**	0,048	246620,7 +/- 1092,4
106 876 001	106 877 000	163	97	0,000	**0,268**	6536900 +/- 133,9

Table S2. Candidate inversion breakpoints. The top table shows regions in which reads either on the X or X* (in bold) behave like in the vicinity of a standard inversion breakpoint (figure 2a). The "same strand reads" column gives the fraction of reads which mates map on the same strand. The bottom table shows regions in which reads follow the second pattern tested to detect inversions (figure 2b).

Discussion

La souris naine africaine est un modèle biologique remarquable de par son déterminisme du sexe atypique. Au cours des trois années de recherche qui ont constitué ma thèse, je me suis attelé à essayer de comprendre les causes ultimes de l'évolution de ce système et d'en identifier les conséquences. C'est la première fois qu'un mammifère thérien avec un déterminisme sexuel non conventionnel est étudié avec autant de détail, et outre les avancées réalisées dans la compréhension de ce système si particulier, les résultats obtenus nous en apprennent plus sur l'évolution des systèmes de déterminisme sexuel en général, et en particulier sur les systèmes polygéniques.

Le suivi de la reproduction et des traits d'histoire de vie des souris dans l'élevage nous ont permis de mettre en évidence que les femelles X*Y produisent plus de descendants au cours de leur vie que les femelles XX et XX* (manuscrit 1) et que mâles ne transmettent pas leurs chromosomes sexuels de manière mendélienne : les mâles appariés à des femelles XX ou XX* transmettent leur chromosome Y à 4/5 de leurs descendants, et ceux en couple avec des femelles X*Y voient au contraire leur chromosome X transmis plus fréquemment, à presque 2/3 de leur progéniture (manuscrit 2). Ces observations ont été reliées à deux hypothèses développées dans la littérature pour expliquer des transitions d'un système de déterminisme à un autre, l'hypothèse d'avantage sélectif de la mutation (Bull & Charnov, 1977) et l'hypothèse de la modification du déterminisme du sexe en réponse à une distorsion de transmission des chromosomes sexuels (Kozielska et al. 2010). En prenant ces observations en compte, une approche théorique nous a permis de proposer des scénarios pour expliquer l'évolution de ce système particulier. Les résultats montrent que les deux facteurs (différences de succès reproducteur chez les femelles et bais de transmission des chromosomes sexuels chez les mâles) sont impliqués dans le maintien du chromosome X*, et que l'un ou l'autre aurait pu être à l'origine de son émergence (manuscrit 2). La distorsion de transmission des chromosomes sexuels mâle dépendante du génotype de la femelle, phénomène décrit pour la première fois à notre connaissance, a fait l'objet d'analyses plus poussées, qui ont révélé que le biais de transmission avait lieu dans les voies génitales des femelles avant la fécondation (Manuscrit 3). Ces résultats ouvrent des pistes de recherche pertinentes pour mieux comprendre les mécanismes sous tendant les distorsions de ségrégation chez les mammifères. La comparaison des mâles et des trois types de femelles a mis en évidence que les femelles X*Y sont de « vraies » femelles d'un point de vue morphologique et anatomique (Manuscrit 4), mais qu'elles

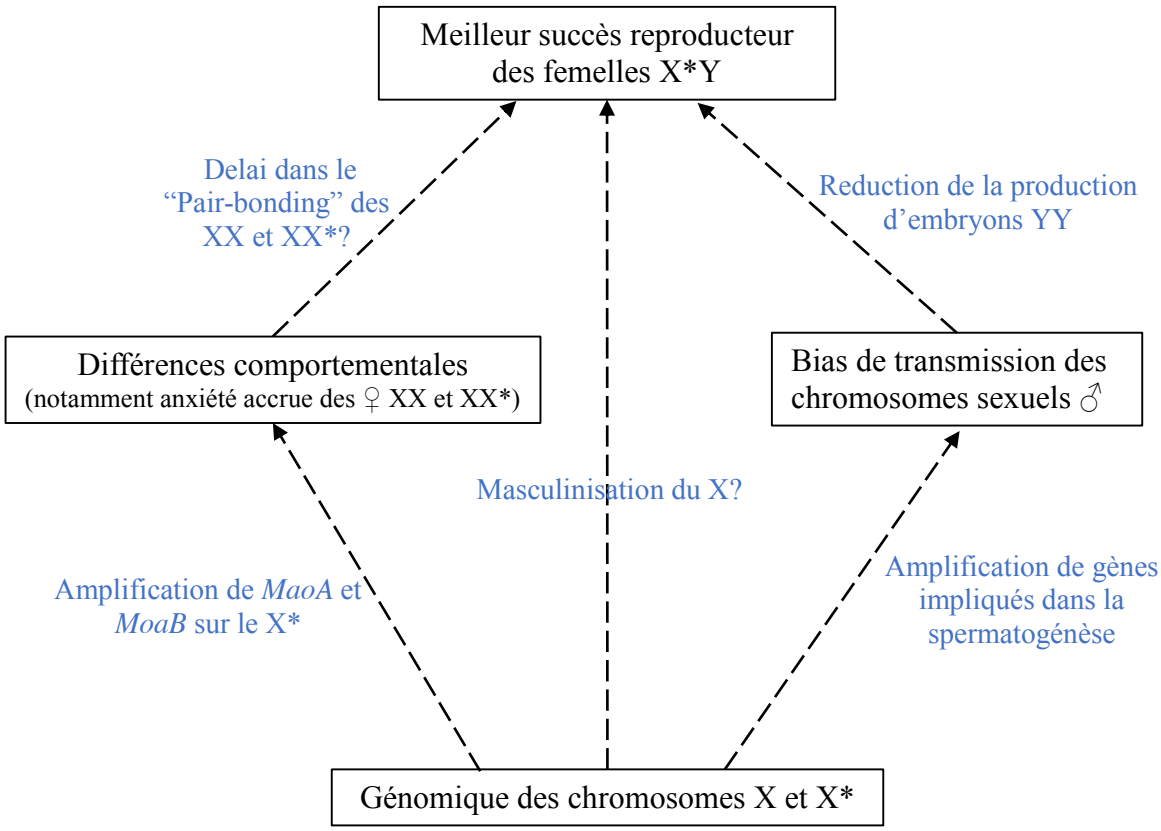


Figure 1 : Mise en relation des résultats obtenus avec les différentes approches.

se distinguent des femelles XX et XX* au niveau de plusieurs traits comportementaux, elles sont plus agressives et se rapprochent plus des mâles en terme d'anxiété notamment (Manuscrit 5). Finalement, la comparaison de la séquence et de la structure des chromosomes X et X* suggère qu'ils ont en large partie arrêté de recombiner et se sont mis à diverger (Manuscrit 6).

Chaque résultat étant discuté dans le détail dans les manuscrits associés, l'objectif de cette discussion est plutôt de faire un point sur la complémentarité entre les différentes approches employées, de dégager des conclusions intégratives plus générales, et de proposer des perspectives de recherche pour approfondir ces travaux.

« Le tout est plus que la somme de ses parties »

L'avantage d'avoir utilisé une approche pluridisciplinaire pour aborder la question des causes et des conséquences de l'évolution d'un système de déterminisme du sexe polygénique chez la souris naine africaine peut être synthétisé en détournant la maxime utilisée en systémique : « le tout est plus que la somme de ses parties ». On peut en effet mettre en relation les résultats obtenus grâce aux différentes approches et voir émerger de nouvelles conclusions et hypothèses. Cette expression est déjà bien illustrée par la complémentarité entre les études empirique sur les traits d'histoire de vie et le sex-ratio et l'approche théorique, qui ont permis de dégager des grands scénarios pour expliquer l'évolution du système. D'autres résultats peuvent être mis en relation de manière similaire (figure 1). Ainsi, on peut mettre en parallèle les résultats concernant les traits d'histoire de vie des femelles et ceux concernant leur comportement, qui suivent le même patron (XX = XX* ≠ X*Y). Beaucoup de femelles XX et XX* ne se reproduisent pas, et celles qui le font mettent en moyenne 20 jours de plus que les femelles X*Y à avoir leur première portée. Le fait que les femelles XX et XX* ont des caractères sexuels primaires identiques aux femelles X*Y suggèrent qu'elles non pas de problèmes de fertilité. Ces différences pourraient par contre être expliquées par les spécificités comportementales des femelles XX et XX*, notamment leur anxiété accrue, qui affectent sans doute les interactions entre mâle et femelle et pourrait entraver le « pair-bonding »[11]. Le fait que ces femelles ont un succès de reproduction inférieur aux femelles X*Y est certainement en partie lié à leurs différences comportementales.

[11] En quelque sorte la tolérance et l' « attachement » des deux partenaires d'un couple.

Toujours concernant les traits d'histoire de vie, il a été montré que les femelles X*Y donnent naissance à des portées plus grandes que les femelles XX et XX*. Cette différence est liée à un taux d'ovulation plus fort chez les premières, mais est sans doute également renforcée par l'évolution du distorteur de transmission des chromosomes sexuels mâles favorisant la transmission de leur chromosome X dans les croisements avec les femelles X*Y. Sachant que ce biais de transmission résulte d'un mécanisme en amont de la fécondation, 18% d'embryons YY sont formés tout au plus (contre 25% si la transmission était mendélienne et 40% si le biais de transmission était similaire à celui observé dans les croisement avec des femelles XX et XX*).

Les résultats de l'approche génomique peuvent également être mis en relation avec des observations faites avec les autres approches. Nous avons notamment pu mettre en évidence l'amplification sur le chromosome X de quelques gènes (en accord avec les données de aCGH), dont deux bien connus des neurobiologistes pour leur effet sur le comportement : *MaoA* et *MaoB*. Il a été montré chez la souris qu'une déficience du premier entrainait une augmentation de l'agressivité (Cases et al. 1995) et que l'absence du second provoquait chez les souris une désinhibition et une réduction de l'anxiété (Bortolato et al. 2009). En admettant que la différence en nombre de copies de ces gènes sur le X et le X* reflète leur niveau d'expression, ils pourraient expliquer l'agressivité plus importante et la moindre anxiété des femelles X*Y. Cette hypothèse est compatible avec le fait que les femelles XX* montrent un niveau d'agressivité intermédiaire, mais n'est pas suffisante pour expliquer pourquoi ces femelles semblent tout aussi anxieuses que les femelles XX. Il faut peut-être chercher une explication du côté de l'interaction de ces gènes avec le chromosome Y. Il a en effet été montré que le gène *Sry* est un activateur de *MaoA* (Wu et al. 2009), or *Sry* est exprimé dans le cerveau des femelles X*Y... Dans tous les cas, *MaoA* et *MaoB* sont de bons candidats pour expliquer les différences observées et il sera intéressant de mesurer les niveau d'expression de ces deux gènes dans le cerveau des mâles et des trois types de femelles[12] pour estimer le potentiel impact de ces gènes sur le comportement de nos souris.

D'autre part, l'apparente masculinisation du chromosome X (amplification de plusieurs gènes impliqués dans la fonction mâle) vient aussi questionner nos interprétations quant aux différences de succès reproducteur des trois types de femelles. Il reste indéniable

[12] Nous possédons d'ores et déjà des amorces pour réaliser des PCR quantitatives.

que les femelles X*Y chez la souris naine africaine ne sont pas lésées par les problèmes de fertilité normalement associées à la présence d'un unique X et d'un Y chez un femelle (Vernet et al. 2014), mais il est également envisageable que les femelles XX et XX* subissent des effets négatifs associé à la modification de la trajectoire évolutive du chromosome X. Alors que dans un système de déterminisme XY classique, le chromosome X passe plus de temps dans un contexte femelle que dans un contexte mâle, dans un système polygénique tel que celui trouvé chez la souris naine africaine, c'est le contraire, et ces conditions pourraient favoriser la fixation de mutations à effet sexuellement antagoniste favorable pour les mâles, et au contraire néfaste pour les femelles portant ce chromosome.

Finalement, cette amplification de gènes impliqués dans la spermatogénèse pourrait avoir une autre explication. Les conflits génomiques entre les chromosomes sexuels sont d'important moteurs de l'évolution des chromosomes sexuels (Soh et al. 2014). Sur le chromosome X de la souris, il existe de nombreux gènes en multiples copies exprimé après la méiose dans les testicules (Mueller et al. 2008). Idem sur le Y, plusieurs gènes de la partie non recombinante mais possédant des homologues sur le X sont massivement amplifiés (Soh et al. 2014). Il a été proposé que ces amplifications résultent de conflits génomiques entre les X et le Y (revue dans Bachtrog 2014). Par exemple, il a été montré que l'amplification des gènes à effet post-méiotique *Slx* et *Sly* (respectivement sur le X et sur le Y) résulte d'un conflit génomique dont l'objet est la transmission des chromosomes sexuels mâles. Dans les souches sauvages de *Mus musculus* et *domesticus*, le ratio du nombre de copies de deux gènes est équilibré, et il est déséquilibré dans certaines souches de laboratoires. Lorsque ce ratio est en faveur de *Sly*, les mâles transmettent leur chromosome Y plus fréquemment (à cause de malformations des spermatozoïdes portant le X), et réciproquement (Cocquet et al. 2012). Ainsi, il est envisageable que les gènes amplifiés sur les chromosomes sexuels de la souris naine africaine soient impliqués dans des conflits génomiques et pourquoi pas dans les biais de transmission des chromosomes sexuels des mâles.

Qui de la poule ou de l'œuf… ?

La combinaison des données récoltées dans l'élevage et l'étude théorique nous a permis de dégager différents scénarios évolutifs pouvant expliquer l'émergence et le maintien du chromosome X*. Le premier scénario est en accord avec les études théoriques qui prédisent qu'une modification du déterminisme du sexe peut se produire en réponse à

l'invasion d'un élément génétique égoïste affectant la transmission des chromosomes sexuels (Kozielska et al. 2010). Chez la souris naine africaine, le X* aurait pu apparaitre suite à l'invasion du distorteur de transmission du chromosome Y observé dans les croisements XY x XX. Alternativement, nos modèles montrent qu'un second scénario est possible. Dans ce scenario, le bais de transmission des chromosomes sexuels mâles serait apparu après l'invasion du X*. Cela nécessite toutefois que les femelles X*Y aient eu un avantage reproductif dès l'émergence de ce chromosome féminisant (Manuscrit 2). Les données dont nous disposons actuellement ne permettent pas de trancher définitivement entre ces deux scénarios. Cependant, le fait que les inversion de sexe ont des conséquences dramatiques sur la fertilité des souris domestiques et d'autres mammifères (revue dans Quinn & Koopman 2012) rendent le second scénario moins probable. Au contraire, dans le 1er scénario, suite à l'invasion d'un Y distorteur, le système polygénique est tolérant à une fertilité réduite des femelles X*Y (Une fitness relative de 25% par rapport aux femelles XX et XX* aurait été suffisant pour maintenir le X* si celui-ci était apparu en réponse à un élément génétique égoïste induisant un ratio de transmission des chromosomes sexuels mâles de l'ordre de 80:20 (Y:X)). Ainsi, il est plus parcimonieux de faire l'hypothèse que les femelles X*Y aient eu à l'origine une fertilité réduite et qu'elles aient acquis un avantage progressivement, au cours du million d'années qui sépare ces observations de l'apparition du X*, par le biais notamment d'une sélection femelle spécifique.

Afin de vérifier ces hypothèses, il serait intéressant d'analyser la fertilité des femelles X*Y ainsi que le ratio de transmission des chromosomes sexuels des mâles dans d'autres populations de la souris naine africaine, et en fonctions des résultats obtenus, il sera peut-être possible d'ordonner chronologiquement l'apparition du X*, des différences de succès reproducteur entre femelles et des distorteurs de transmission.

Femelles X*Y, des femelles comme les autres ?

Moore et Roberts ont récemment émis l'hypothèse qu'on pourrait voir émerger chez les espèces à déterminisme polygénique plusieurs « classes » au sein d'un même sexe : des individus avec le même sexe primaire mais caractères sexuels secondaires différents (Moore & Roberts 2013).

Chez la souris naine, les femelles X*Y sont à première vue des femelles tout ce qu'il y a de plus banales. Elles sont indistinguables des autres types de femelles dans l'élevage (ce qui nous contraint d'ailleurs de passer par une étape de génotypage

moléculaire pour déterminer leur complément en chromosomes sexuels) et présentent des caractéristiques morphologiques et anatomiques identiques aux autres femelles, sans aucun signe visible de masculinisation. Cependant le complément en chromosomes sexuels qu'arbore une femelle à bel est bien des conséquences sur son phénotype : les femelles X*Y se distinguent des autres femelles en terme de fertilité, de comportement, et les trois types de femelles produisent toutes des mâles en proportions différentes (environ 80% pour les XX, et respectivement 37% et 42% pour les XX* et X*Y). Ces différences phénotypiques ne sont pas étonnantes lorsqu'on considère que les chromosomes sexuels des mammifères sont un hot-spot pour les gènes impliqués dans la reproduction, et que le chromosome X porte un excès de gènes impliqués dans la différentiation sexuelle, le neuro-développement et la cognition (Hurst and Randerson 1999; Carruth et al. 2002; Skuse 2005, 2006). Ces différences ont certainement des conséquences écologiques importantes.

Les conditions dans lesquelles nous avons fait ces observations (au sein de notre élevage) sont rigoureusement contrôlées, les adultes sont placés dans des cages en couple lors de leur maturité sexuelle et n'interagissent plus directement avec d'autres congénères. Il est probable que les différences phénotypiques entre les trois types de femelles soient plus marquées encore en milieu naturel, et que les femelles adoptent des stratégies de reproduction alternatives (Gross 1996). En retour, les femelles doivent être soumisses à des pressions de sélection différentes, potentiellement conflictuelles. L'évolution du génome de la souris naine pourrait donc être soumis à une pression de sélection « génotype-spécifique », au même titre que les génomes des organismes à reproduction sexuée sont soumis à la sélection sexe-spécifique (Wright and Mank 2013).

A défaut d'observer les souris sur le terrain, il serait intéressant de les placer dans un environnement permettant plus d'interactions, comme une cage à population. On pourrait ainsi observer dans le détail les comportements des femelles (e.g. interactions inter- et intra-sexuelles, territorialité etc) et mettre à l'épreuve cette idée de stratégies de reproduction alternatives.

Nos observations sur la souris naine africaine sont la première confirmation de l'hypothèse de Moore et Roberts, et révèlent la pertinence d'étudier dans le détail d'autre espèces possédant des systèmes de déterminisme polygénique, notamment des systèmes ou les chromosomes sexuels ancestraux ne sont pas différenciés afin de voir si on y retrouve aussi ces différentes « classes » au sein d'un même sexe.

Quand les conflits deviennent une guerre

Les différents systèmes de déterminisme du sexe ne sont pas tous égaux face aux conflits génomiques. Dans les systèmes de déterminisme hétérogamétique par exemple, à cause de leurs patrons de transmission particuliers, les chromosomes sexuels sont un véritable champ de bataille pour les éléments génétiques égoïstes affectant le sex-ratio (Burt and Trivers 2006). La souris naine africaine, avec son système polygénique, n'est pas en reste : plusieurs éléments génétiques affectent le sex-ratio : Le chromosome X*[13], qui induit la production d'un excès de femelles, et au moins un ou deux éléments génétiques affectant de manière forte le ratio de transmission des chromosomes sexuels des mâles. De manière intéressante, la présence de distorteurs de transmission des chromosomes sexuels est récurrente chez les mammifères à déterminisme polygénique. Chez les femelles X*Y de *Myopus schisticolor*, le Y est éliminé de la lignée germinale par un mécanisme de double non-disjonction des chromosomes dans l'ovaire fœtal (Winking et al. 1981), et une faible distorsion de transmission du Y mâle (0,54-0,59) a été trouvé chez *Dicrostonyx torquatus* (Gileva 1987). Comment expliquer que ces systèmes semblent être propices à l'accumulation de distorteurs de transmission sachant qu'ils sont rares chez les mammifères avec un déterminisme conventionnel ? Plusieurs explications sont proposées. D'une part, il est possible que la relative « jeunesse » de ces systèmes soit en cause (pour rappel, le X* de *Mus minutoides* est apparu il y a moins d'1 million d'années), et que les conflits n'aient pas encore pu être résolus de manière plus élégante que part l'invasion de multiples distorteurs (les éléments génétiques affectant la transmission des chromosomes sexuels mettent en effet une pression de sélection forte sur les compartiments génomiques lésés, ce qui fait que n'importe quel moyen pour réduire leur effet sera rapidement sélectionné). Les génomes de ces espèces font « avec les moyens du bord », peut-être jusqu'à ce qu'un suppresseur de la distorsion initiale évolue. De plus, il est également envisageable que ces systèmes soient enclins à l'accumulation d'éléments génétiques égoïstes, car l'apparition d'un troisième chromosome sexuel augmente le nombre de compartiments génomiques pouvant entrer en conflit. D'autre part, les systèmes de déterminisme polygénique pourraient tout simplement être plus tolérants envers les distorteurs de transmission des chromosomes sexuels. L'apparition du X* a modifié les patrons de transmission des chromosomes X et Y, et biaise le sex-ratio des portées des femelles XX et XX* mais aussi

[13] Les chromosomes féminisants qu'on retrouve chez plusieurs espèces de mammifères sont d'ailleurs considérés comme des éléments génétiques égoïstes par Burt & Trivers

de la population, modifiant les pressions de sélection s'exerçant sur les conflits génomiques. Par exemple, l'intérêt des autosomes s'aligne sur celui des chromosomes sexuels (le Y dans les croisements avec des femelles XX et XX* et le X dans les croisements avec des femelles X*Y) : produire plus de mâles. Un suppresseur des distorteurs de transmission n'a donc pas de raison d'apparaitre sur un autosome (et notre étude théorique montre que les éléments distorteurs pourraient même être portés par un autosome ; voir manuscrit 2).

Quel que soit la raison de la prépondérance des distorteurs de transmission chez les espèces de mammifères à déterminisme atypique, ils font d'excellents modèles pour étudier les conflits génomiques et investiguer les bases génétiques des distorsions de transmission des chromosomes sexuels.

Quel avenir pour ce système ?

Globalement, les déterminismes du sexe atypiques chez les mammifères se trouvent uniquement dans les branches terminales de la phylogénie, et il n'existe pas de lignées complètes avec un système non-conventionnel. Il est cependant probable que des déterminismes bizarres soient apparus et se soient maintenus à de nombreuses reprises au cours des quelques 150 millions d'années d'évolution des mammifères. Pourquoi ne sont-ils pas plus nombreux? Deux cas de figures peuvent être envisagés : (i) soit une extinction systématique des espèces concernées, mais pour l'heure, la raison pour laquelle ces systèmes seraient forcément voués à l'échec n'est pas tout à fait claire, surtout quand on voit la longévité du X* chez *Mus minutoides*. (ii) soit un retour systématique au déterminisme classique. Dans un futur proche, nous aurons surement accès à des données génomiques pour de nombreux mammifères, et il sera intéressant de tester cette hypothèse en comparant en détail leurs chromosomes sexuels. Ceci est très spéculatif, mais chez certaines espèces, ils pourraient porter les stigmates d'un bref passage par un mode de déterminisme atypique, à l'instar du chromosome « dot » de la drosophile, un autosome qui était ancestralement un chromosome X différentié (Vicoso and Bachtrog 2013).

Un retour à un système hétérogamétique classique semble compliqué pour la souris naine africaine. Un suppresseur de l'action féminisante du X* pourrait évoluer sur le Y ou un autosome, mais dans l'état actuel, la disparition du X* serait synonyme de la production de 80% de mâles dans toutes les portées, qui pourrait rapidement mener à l'extinction de la population concernée. En fait, si un des trois chromosomes sexuels venait à disparaitre, il

y a des chances que ce soit le Y. Plusieurs espèces de mammifères ont perdu leur Y, dont le rat Japonais *Tokudaia osimensis* chez qui mâles et femelles ont un complément X0 (Honda & Itoh 1977). Le déterminisme du sexe de cette espèce n'est plus dépendant de *Sry* (Soullier et al. 1998), et plusieurs gènes du Y indispensables à la fonction mâle ont été transloqués sur le X (Kuroiwa et al. 2010), ce qui a sans doute précipité sa perte. Si de telles translocations se produisaient chez la souris naine, elle pourrait aussi perdre son Y (et peut-être par la même occasion se débarrasser des distorteurs de transmission ?). Le déterminisme du sexe serait alors entièrement assuré par le X* : les individus X*0 serait femelles et X0 mâles. Curieusement, un proche parent de *Mus minutoides*, *Mus triton*, possède un système X0/X0 (Jotterand-Bellomo 1988), on ne connait rien de la manière dont le sexe est déterminé chez cette espèce, mais il est possible qu'elle soit passée par une étape intermédiaire de déterminisme polygénique avant de perdre son chromosome Y.

Si le système polygénique se maintien longtemps dans l'état, que peut-on prédire quant à l'évolution des chromosomes sexuels? Le chromosome X pourrait se masculiniser (certains résultats de la comparaison du X et du X* vont dans ce sens), du fait qu'il passe plus dans temps dans un contexte mâle que femelle. Il est difficile de faire des prédictions pour le chromosome Y dans la mesure où son contenu en gènes est spécialisé à l'extrême. Comme expliqué plus haut, certains de ses gènes pourraient être transloqués sur le X. Finalement, malgré sa transmission limitée à la lignée femelle, il est peu probable qu'on voit le X* dégénérer comme ça peut arriver au Y ou au W dans un système hétérogamétique classique (tout du moins pas au même rythme). Les gènes spécialisés dans la fonction mâle ne sont bien sûr plus sous sélection et risquent de devenir des pseudogènes, mais il porte aussi un excès de gènes impliqués dans des fonctions reproductives femelle et cognitives, qui sont sous forte pression de sélection purifiante chez les femelles X*Y car ils n'ont pas d'homologue sur le Y. Il pourrait par contre se féminiser en accumulant des allèles et gènes favorables pour les femelles.

Comme l'a fait remarquer Jenny Graves (2008), l'étude de systèmes de déterminisme du sexe « alternatifs » est importante et ils ont notamment grandement contribué à la compréhension des déterminismes « classiques ». *Mus minutoides*, avec son système de déterminisme du sexe à trois chromosomes sexuels, s'inscrit dans cet esprit. En plus de la contribution à la meilleure compréhension de son curieux mode de déterminisme du sexe, ce projet a permis d'en apprendre plus sur les contraintes s'appliquant sur les systèmes chromosomiques anciens, et d'éclaircir les conséquences phénotypiques et génomiques de la présence de plusieurs gènes influençant le déterminisme du sexe au sein d'un même génome.

La souris naine africaine est une espèce remarquable à bien des égards, et a encore de nombreux secrets à nous livrer. A travers ce manuscrit, j'espère avoir réussi à vous montrer l'intérêt de ce modèle biologique qui a de beaux jours devant lui.

Annexes

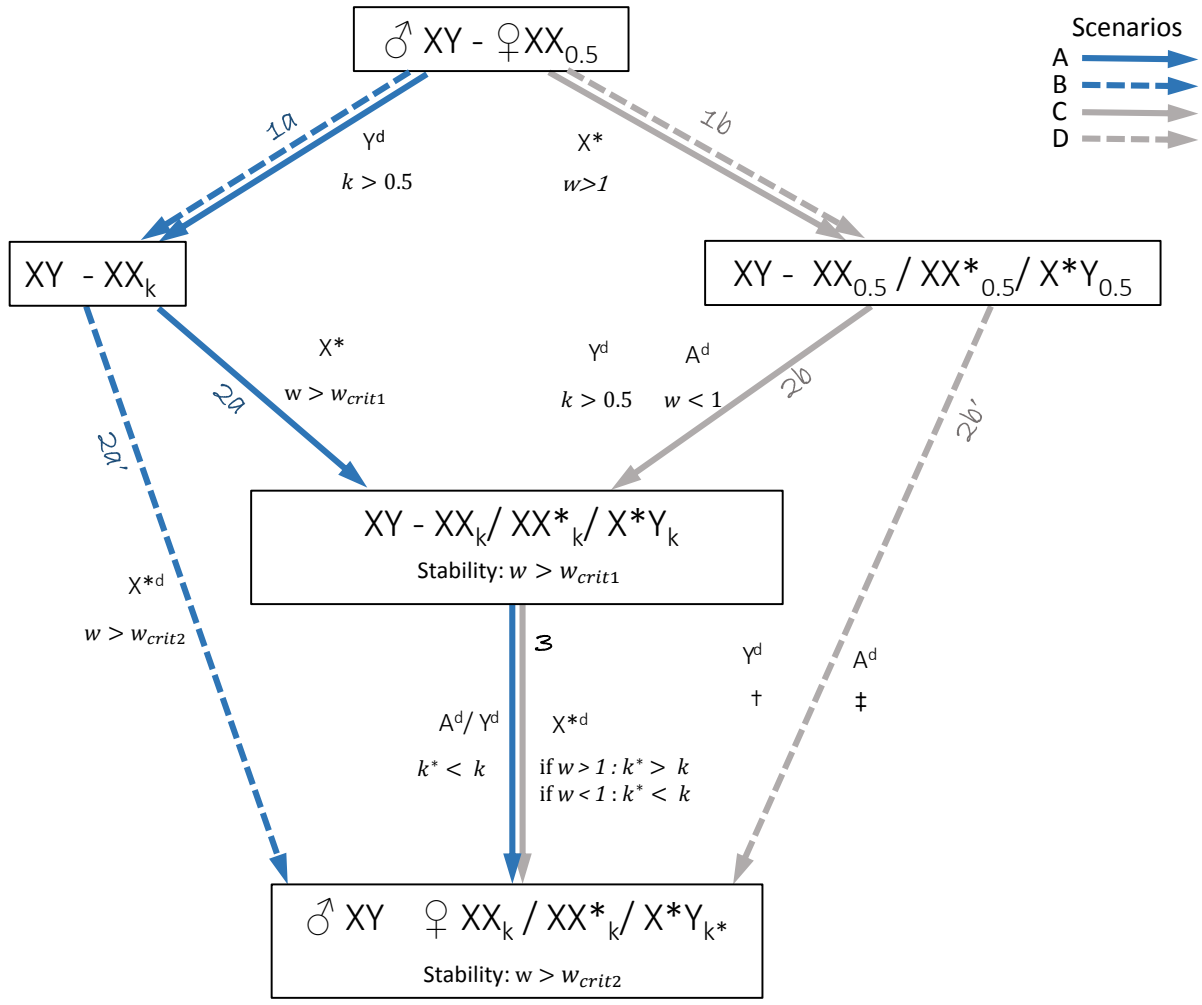


Figure 1: Paths to the evolution of the unusual sex determination of *Mus minutoides*. Each box represents a stable state in which sex determination is given (either standard male heterogamety, or a polygenic X/X*/Y sex determination system). The subscripts (0.5, k, k*) refer to the transmission ratio of male sex chromosomes in each cross. And the condition for stability of the state is given under the previous information. Arrows represent steps (1a, 2a) leading from one state to another. The type/colour of the arrow denote the different scenarios described in the text. At each arrow corresponds an invasion analysis, and next to the arrow is (i) the chromosome that harbours the mutation considered (A for autosome), the superscript informs on the type of mutation (*: feminizing mutation, d: transmission distorter), (ii) the condition for invasion. $w_{crit1} = \frac{1-k}{k}$, $w_{crit2} = \frac{1-k}{k} * \frac{1-k}{\frac{1}{2}-k^*(1-\frac{1}{2k})}$, † : $k \geq \frac{1}{2}\frac{w^2+1}{2w^2-w+1}$ and $k^* \leq \frac{1}{2}\frac{4kw^2-w^2+2k-2kw-1}{2w^2-2kw}$ or $w < 1+\sqrt{2}$ and $k \geq \frac{1}{2}\frac{w^2+1}{2w^2-w+1}$, ‡: see appendix B.

Appendix for manuscript 2

Here we describe the theoretical models discussed in the main text.

Appendix A: Stability analysis: maintenance of the X*

In the different scenarios that are proposed to explain the evolution of the unusual sex determination system of the African pygmy mouse, there are three equilibria in which the X* is present (figure 1). At the first equilibrium: *eq1* (XY - $XX_{0.5}$ /$XX^*_{0.5}$ /$X^*Y_{0.5}$), there is no transmission distortion of male sex chromosomes, at the second: *eq2* (XY - XX_k/XX^*_k/X^*Y_k), males transmit their Y chromosome at a ratio k, regardless of the genotype of the female: the transmission distortion of male sex chromosomes (TDMSC) is unconditional. At the third equilibrium: *eq3*, TDMSC is conditional: strength of drive is k in crosses with XX and XX* females and k^* in crosses with X*Y females (XY - XX_k /XX^*_k /$X^*Y_{k^*}$). The aim of the stability analysis is to define the conditions for stability of these three equilibria, in other words, determine the set of parameters allowing the maintenance of the X*, following methods described in Otto & Day (2007).

The models describing the three equilibria are simplifications of a more general model considering one type of male and three types of females: XX, XX* and X*Y, in number (f_1, f_2, f_3), and in which transmission ratio of male sex chromosomes can be different in the three types of crosses (k_{XX} , k_{XX^*} , k_{X^*Y}), and females have different fitness ($w_{XX} = 1$, w_{XX^*} , w_{X^*Y}).

The dynamics of the model is given by the recursions:

$$\begin{bmatrix} f_1 \\ f_2 \\ f_3 \end{bmatrix}_{(t+1)} = M \begin{bmatrix} f_1 \\ f_2 \\ f_3 \end{bmatrix}_{(t)}$$

With the transition matrix:

$$M = \begin{bmatrix} 1 - k_{XX} & w_{XX^*}\frac{1-k_{XX^*}}{2} & 0 \\ 0 & w_{XX^*}\frac{1-k_{XX^*}}{2} & w_{X^*Y}\frac{1-k_{X^*Y}}{2} \\ 0 & w_{XX^*}\frac{k_{XX^*}}{2} & w_{X^*Y}\frac{k_{X^*Y}}{2} \end{bmatrix}$$

m_{ij} elements describe the number of females of genotype i in the progeny of a female of genotype j.

The study of the eigensystem of the transition matrice provides the conditions for stability: the eigenvector associated to the greatest eigenvalue gives the frequencies at equilibrium, and the stability conditions can be obtained by comparing non-zero eigenvalues (the procedure is detailed for the first equilibrium).

Eq 1. $\boldsymbol{k_{XX} = k_{XX^*} = k_{X^*Y} = 0.5}$ **and** $\boldsymbol{w_{XX} = w_{XX^*} = 1,\ w_{X^*Y} = w}$

$$M_{eq1} = \begin{bmatrix} \frac{1}{2} & \frac{1}{4} & 0 \\ 0 & \frac{1}{4} & \frac{w}{4} \\ 0 & \frac{1}{4} & \frac{w}{4} \end{bmatrix}$$

The two non-zero eigenvalues of the matrix for equilibrium 1 M_{eq1} are $\lambda 1 = \frac{1}{2}$ and $\lambda 2 = \frac{1+w}{4}$. The eigenvector associated to $\lambda 1$ is {1,0,0} (*i.e.* if $\lambda 1$ is the leading eigenvalue, all females are XX) and the one associated to $\lambda 2$ is $\{-\frac{1}{1-w}, 1, 1\}$ (*i.e.* if $\lambda 2$ is the leading eigenvalue, XX, XX* and X*Y females are in relative number $-\frac{1}{1-w}, 1, 1$, or frequency $\frac{1}{2w-1}$, $\frac{w-1}{2w-1}$, $\frac{w-1}{2w-1}$).

If $\lambda 1 > \lambda 2$, X* cannot be maintained at equilibrium, whereas it can be maintained if $\lambda 1 < \lambda 2$. In other words, the system with X* is stable if and only if $w > 1$, meaning that as long X*Y females have a higher fitness than XX and XX* ones, the X* is maintained.

Eq 2. $\boldsymbol{k_{XX} = k_{XX^*} = k_{X^*Y} = k}$ **and** $\boldsymbol{w_{XX} = w_{XX^*} = 1,\ w_{X^*Y} = w}$

$$M_{eq1} = \begin{bmatrix} 1-k & \frac{1-k}{2} & 0 \\ 0 & \frac{1-k}{2} & w\frac{1-k}{2} \\ 0 & \frac{k}{2} & w\frac{k}{2} \end{bmatrix}$$

The two non-zero eigenvalues of M_{eq2} are $\lambda 1 = 1 - k$ and $\lambda 2 = \frac{1}{2}(1 - k + kw)$. According to the eigenvectors, the X* is maintained if $\lambda 1 < \lambda 2 : w > \frac{1-k}{k}$ $(= w_{crit1})$, the threshold value for w. Here, either a higher reproductive success of X*Y females or a driving Y would make the maintenance of the X* possible.

Eq 3. $\boldsymbol{k_{XX} = k_{XX^*} = k,\ k_{X^*Y} = k^*}$ **and** $\boldsymbol{w_{XX} = w_{XX^*} = 1,\ w_{X^*Y} = w}$

$$M_{eq3} = \begin{bmatrix} 1-k & \frac{1-k}{2} & 0 \\ 0 & \frac{1-k}{2} & w\frac{1-k^*}{2} \\ 0 & \frac{k}{2} & w\frac{k^*}{2} \end{bmatrix}$$

The two biggest eigenvalues are $\lambda 1 = 1 - k$ and $\lambda 2 = \frac{1}{4}(1-k+k^*w+\sqrt{1-2k^2+4kw-2k^*-2kk^*w+k^{*2}w^2})$. The system is stable for $\lambda 1 < \lambda 2$ *i.e.* when $w > \frac{1-k}{k} * \frac{1-k}{\frac{1}{2}-k^*(1-\frac{1}{2k})}$ (w_{crit2}). This stability threshold value depends on both on k and k^*: with $k > 0.5$, the second term of w_{crit2} is inferior to 1 if $k > k^*$, and gets smaller as k^* decreases. In other words, the more k is big and k^* small, the more tolerant the system will be to low values of w. With $k < 0.5$, it is the opposite and w_{crit2} will decrease with increasing values of k^*.

Appendix B: Invasion analyses

The invasion analyses for all mutations allowing to go from one equilibrium to another are presented here. In cases where models are non-linear (here when there are several male genotypes and several female genotypes, the method relies on the calculation of the leading eigenvalue of the Jacobian matrix associated with the system of equations for the equilibrium of interest, and is described in Otto and Day (2007).

Invasion of a mutant Y

Step 1a: "Genes inherited in a biased manner can spread in a population without doing anything good for the organism" (Burt & Trivers 2006). A driving Y will spread if $k > 0.5$.

Step 2b: Invasion of an unconditional driving Y in a polygenic system (*eq 1*). The alleles considered are : Y, Y^d (the driving Y), X and X*. There are two types of males: XY and XY^d and four types of females: XX, XX*, X*Y and X^*Y^d, in number m_1, m_2 and $f_1...f_4$ respectively and frequencies y_1, y_2 and $x_1...x_4$. In XY males and all females, transmission ratio of sex chromosomes is mendelian. In XY^d males, the driving allele Y^d has a transmission ratio of k (see table below). Finally, sex reversed females (X*Y and X^*Y^d) have a fertility w.

crosses		offspring genotypes					
male	female	XY	XY^d	XX	XX*	X*Y	X^*Y^d
XY	XX	$\frac{1}{2}$	0	$\frac{1}{2}$	0	0	0
XY	XX*	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0
XY	X*Y	$\frac{1}{4}$	0	0	$\frac{1}{4}$	$\frac{1}{4}$	0
XY	X^*Y^d	0	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	0
XY^d	XX	0	k	1-k	0	0	0
XY^d	XX*	0	$\frac{k}{2}$	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X*Y	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X^*Y^d	0	$\frac{1-k}{2}$	0	$\frac{1-k}{2}$	0	$\frac{k}{2}$

The dynamics of the model can be described by the following recursions:

$$m_1' = \bar{f}\left[y_1\left(\frac{x_1}{2}+\frac{x_2}{4}+\frac{x_3 w}{4}\right)+y_2 x_3 w\frac{1-k}{2}\right]$$
$$m_2' = \bar{f}\left[y_1\frac{x_4 w}{4}+y_2\left(\left(x_1+\frac{x_2}{2}\right)k+x_4 w\frac{1-k}{2}\right)\right]$$
$$f_1' = \bar{f}\left[\left(\frac{y_1}{2}+y_2(1-k)\right)\left(x_1+\frac{x_2}{2}\right)\right]$$
$$f_2' = \bar{f}\left[\left(\frac{y_1}{4}+y_2\frac{1-k}{2}\right)(x_2+(x_3+x_4)w)\right]$$
$$f_3' = \bar{f}\left[\frac{y_1}{4}(x_2+(x_3+x_4)w)\right]$$
$$f_4' = \bar{f}\left[y_1\frac{k}{2}(x_2+(x_3+x_4)w)\right]$$

Where m'_i and f'_i are the number of males of genotype i and females of genotype j at the next generation and $\bar{f} = \sum_i f_i$.

The frequencies at the next generation can be obtained as follows:

$$y'_i = \frac{m'_i}{\sum_j m'_j} \text{ and } x'_i = \frac{f'_i}{\sum_j f'_j}$$

.

To determine the stability of a system with Mendelian transmission of male sex chromosomes in respect to the invasion of an unconditional Y^d, we evaluate the Jacobian J of the system at the equilibrium point where the driving Y is absent (*eq 1*): All males are XY and the frequencies of XX, XX* and X*Y females are given by the eigenvector of the greatest eigenvalue of the transition matrix M_{eq1}: $\hat{y_1} = 1,\ \hat{y_2} = 0,\ \hat{x_1} = \frac{1}{2w-1},\ \hat{x_2} = \hat{x_3} = \frac{w-1}{2w-1},\ \hat{x_4} = 0$.

$$J = \begin{bmatrix} \frac{\delta x'_1}{\delta \hat{x_1}} & \frac{\delta x'_1}{\delta \hat{x_2}} & \frac{\delta x'_1}{\delta \hat{x_3}} & \frac{\delta x'_1}{\delta \hat{x_4}} & \frac{\delta x'_1}{\delta \hat{y_1}} & \frac{\delta x'_1}{\delta \hat{y_2}} \\ \frac{\delta x'_2}{\delta \hat{x_1}} & \frac{\delta x'_2}{\delta \hat{x_2}} & \frac{\delta x'_1}{\delta \hat{x_3}} & \frac{\delta x'_2}{\delta \hat{x_4}} & \frac{\delta x'_2}{\delta \hat{y_1}} & \frac{\delta x'_2}{\delta \hat{y_2}} \\ \frac{\delta x'_3}{\delta \hat{x_1}} & \frac{\delta x'_3}{\delta \hat{x_2}} & \frac{\delta x'_3}{\delta \hat{x_3}} & \frac{\delta x'_3}{\delta \hat{x_4}} & \frac{\delta x'_3}{\delta \hat{y_1}} & \frac{\delta x'_3}{\delta \hat{y_2}} \\ \frac{\delta x'_4}{\delta \hat{x_1}} & \frac{\delta x'_4}{\delta \hat{x_2}} & \frac{\delta x'_4}{\delta \hat{x_3}} & \frac{\delta x'_4}{\delta \hat{x_4}} & \frac{\delta x'_4}{\delta \hat{y_1}} & \frac{\delta x'_4}{\delta \hat{y_2}} \\ \frac{\delta y'_1}{\delta \hat{x_1}} & \frac{\delta y'_1}{\delta \hat{x_2}} & \frac{\delta y'_1}{\delta \hat{x_3}} & \frac{\delta y'_1}{\delta \hat{x_4}} & \frac{\delta y'_1}{\delta \hat{y_1}} & \frac{\delta y'_1}{\delta \hat{y_2}} \\ \frac{\delta y'_2}{\delta \hat{x_1}} & \frac{\delta y'_2}{\delta \hat{x_2}} & \frac{\delta y'_2}{\delta \hat{x_3}} & \frac{\delta y'_2}{\delta \hat{x_4}} & \frac{\delta y'_2}{\delta \hat{y_1}} & \frac{\delta y'_2}{\delta \hat{y_2}} \end{bmatrix} =$$

$$\begin{bmatrix} \frac{4(f_2+f_3w)}{(2f_1+3f_2+2f_3w)^2} & \frac{2f_3w-4f_1}{(2f_1+3f_2+2f_3w)^2} & -\frac{2w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & -\frac{2w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & 0 & -\frac{2(2k-1)(2f_1+f_2)(f_2+f_3w)}{(2f_1+3f_2+2f_3w)^2} \\ -\frac{2(f_2+f_3w)}{(2f_1+3f_2+2f_3w)^2} & \frac{2f_1-f_3w}{(2f_1+3f_2+2f_3w)^2} & \frac{w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & \frac{w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & 0 & -\frac{2(2k-1)(f_2+f_3w)^2}{(2f_1+3f_2+2f_3w)^2} \\ -\frac{2(f_2+f_3w)}{(2f_1+3f_2+2f_3w)^2} & \frac{2f_1-f_3w}{(2f_1+3f_2+2f_3w)^2} & \frac{w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & \frac{w(2f_1+f_2)}{(2f_1+3f_2+2f_3w)^2} & 0 & \frac{2(f_2+f_3w)(2f_1(k-1)+f_2(k-2)-f_3w)}{(2f_1+3f_2+2f_3w)^2} \\ 0 & 0 & 0 & 0 & 0 & \frac{2k(f_2+f_3w)}{2f_1+3f_2+2f_3w} \\ 0 & 0 & 0 & -\frac{w}{2f_1+f_2+f_3w} & 0 & -\frac{2k(2f_1+f_2)}{2f_1+f_2+f_3w} \\ 0 & 0 & 0 & \frac{w}{2f_1+f_2+f_3w} & 0 & \frac{2k(2f_1+f_2)}{2f_1+f_2+f_3w} \end{bmatrix}$$

A rare Y^d chromosome will spread if the equilibrium is locally unstable, *i.e.* if the leading eigenvalue of the Jacobian is larger than 1, and assuming that the condition for stability of equilibrium *eq 1* ($w > 1$) is satisfied.

The leading eigenvalue of the Jacobian is $\lambda = \frac{\sqrt{k\left(k(w+1)^2 + 2(w-1)w\left(w^2+1\right)\right)} + kw + k}{w^2+1}$.
$\lambda > 1$ for $k > \frac{1}{2}$, meaning that a Y^d will invade if it favors its own transmission.

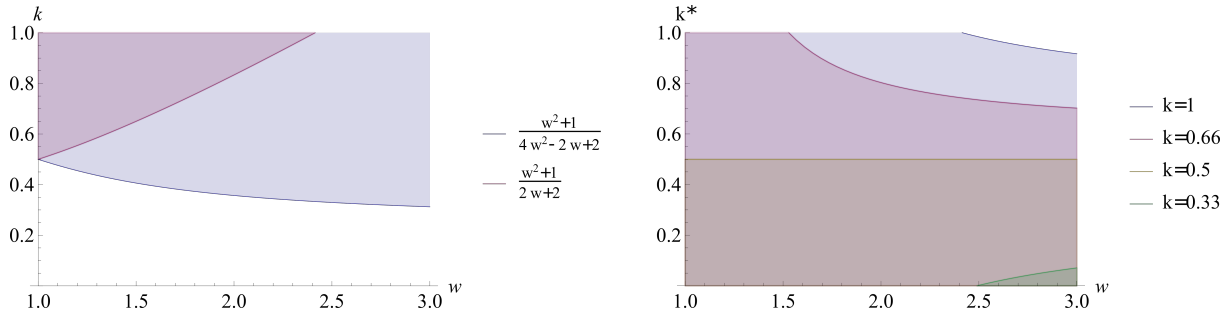
Step 2b': Invasion of a conditional driving Y in a polygenic system (*eq 1*). The alleles considered are : Y, Y^d (the driving Y), X and X*. There are two types of males: XY and XY^d and four types of females: XX, XX*, X*Y and X^*Y^d, in number m_1, m_2 and $f_1...f_4$ respectively. In males and females not bearing the Y^d, transmission ratio of sex chromosomes is Mendelian. In a male context, the Y^d chromosome has a transmission ratio of k, and in a female context, it has an influence on the transmission ratio of male sex chromosomes, which transmission ratio becomes k^* (it overrides the male effect in XY^d x X^*Y^d crosses, see table below). Finally, sex reversed females (X*Y and X^*Y^d) have a fertility w.

crosses		offspring genotypes					
male	female	XY	XY^d	XX	XX*	X*Y	X^*Y^d
XY	XX	$\frac{1}{2}$	0	$\frac{1}{2}$	0	0	0
XY	XX*	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0
XY	X*Y	$\frac{1}{4}$	0	0	$\frac{1}{4}$	$\frac{1}{4}$	0
XY	X^*Y^d	0	$\frac{1-k^*}{2}$	0	$\frac{1-k^*}{2}$	$\frac{k^*}{2}$	0
XY^d	XX	0	k	1-k	0	0	0
XY^d	XX*	0	$\frac{k}{2}$	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X*Y	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X^*Y^d	0	$\frac{1-k^*}{2}$	0	$\frac{1-k^*}{2}$	0	$\frac{k^*}{2}$

In a similar way to step 2b, the recursion equations were written and the Jacobian matrix of the system was evaluated at the equilibrium point *eq 1*. Again, a rare Y^d chromosome will spread if the leading eigenvalue of the Jacobian is larger than 1, and assuming that the condition for stability of equilibrium *eq 1* ($w > 1$) is satisfied.

The leading eigenvalue of the Jacobian is $\lambda = \frac{k+kw+\sqrt{k(k(1+w)^2-4(-1+k^*)(-1+w)w(1+w^2))}}{1+w^2}$

$\lambda > 1$ for (i) $k \geq \dfrac{w^2+1}{2+2w}$, regardless of the value of k^*, as long as $w < 1+\sqrt{2}$ ($\sim$2.41) or (ii) $\dfrac{w^2+1}{4w^2-2w+2} < k \leq \dfrac{w^2+1}{2+2w}$ and $k^* \geq \dfrac{1}{2}\dfrac{4kw^2-w^2+2k-2kw-1}{2w^2-2kw}$.



These plots show the space of parameters allowing the invasion of a Y^d chromosome at step 2b. In the first plot is shown in pink the values of k allowing the spread of the mutation for condition (i), and in blue for condition (ii). On the second plot is shown the value of k^* (for different values of k) allowing the spread of the mutation for condition (ii).

Step 3: Invasion of a TDMSC on the Y specific to XY x X*Y crosses in a system with a preexisting unconditional TDMSC (*eq 2*). The alleles considered are : Y, Y^d (the novel driving Y), X and X*. There are two types of males: XY and XY^d and four types of females: XX, XX*, X*Y and X^*Y^d, in number $m1$, $m2$ and $f1...f4$ respectively. In all females, transmission ratio of sex chromosomes is Mendelian. In males, transmission ratio of sex chromosomes is k, except in crosses with X^*Y^d females, where transmission ratio becomes k^* (see table below). Finally, sex reversed females (X*Y and X^*Y^d) have a fertility w.

crosses		offspring genotypes					
male	female	XY	XY^d	XX	XX*	X*Y	X^*Y^d
XY	XX	k	0	$1-k$	0	0	0
XY	XX*	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	$\frac{k}{2}$	0
XY	X*Y	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	$\frac{k}{2}$	0
XY	X^*Y^d	0	$\frac{1-k}{2}$	0	$\frac{1-k}{2}$	$\frac{k}{2}$	0
XY^d	XX	0	k	1-k	0	0	0
XY^d	XX*	0	$\frac{k}{2}$	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X*Y	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	$\frac{k}{2}$
XY^d	X^*Y^d	0	$\frac{1-k^*}{2}$	0	$\frac{1-k^*}{2}$	0	$\frac{k^*}{2}$

In a similar way to step 2b, the recursion equations were written and the Jacobian matrix of the system was evaluated at the equilibrium point *eq 2*. The frequencies of males and females at this equilibrium are given by the eigenvector of the greatest eigenvalue of the transition matrix M_{eq2}: $\hat{y_1} = 1,\ \hat{y_2} = 0,\ \hat{x_1} = \frac{(1-k)^2}{k(k+w-1)},\ \hat{x_2} = \frac{(1-k)(k+kw-1)}{k(k+w-1)}, \hat{x_3} = \frac{k+kw-1}{k+w-1},\ \hat{x_4} = 0$, the condition for stability is $w > \frac{1-k}{k}$.

The leading eigenvalue of the Jacobian is $\lambda = -\frac{1-2k+k^*+kw-k^*w+\sqrt{\phi}}{2(-1+k)(1-w+k(-1+2w+w^2))}$ with $\phi = (-1+k)((-1+k)(1+k(-1+w))^2+4(-1+k^*)w(-1+k+kw)(1-w+k(-1+2w+w^2)))$.

$\lambda > 1$ for $k^* < k$, so a rare Y^d will spread if it reduces the transmission ratio of male Y chromosomes in crosses with X*Y females.

Invasion of a mutant autosome

The study of the invasion of a TDMSC harbored by an autosome requires the introduction in models of a second locus, which harbours a standard non-distorting a allele and a dominant distorter A allele. This locus is independent from the sex chromosome locus, which implies that for each sex chromosome complement, there are three genotypes (e.g. XXaa, XXAa, XXAA).

Step 2b: Invasion of an autosome causing unconditional drive of male sex chromosomes in a polymorphic system (*eq 1*). At the sex determining locus, the alleles considered are: Y, X and X*, and at the autosomal locus: a and the driving A. There are three types of males: XYaa, XYAa and XYAA and nine types of females: XXaa, XXAa, XXAA, XX*aa, XX*Aa, XX*AA,

X*Yaa, X*YAa, X*YAA in number $m_1...m_3$ and $f_1...f_9$ respectively and frequencies $y_1...y_3$ and $x_1...x_9$ respectively. In XYaa males and all females, transmission ratio of sex chromosomes is mendelian. In XYAa and XYAA males, the Y chromosome has a transmission ratio of k (see table below).Sex reversed females (X*Yaa, X*YAa and X*YAA) have a fertility w.

crosses		offspring genotypes											
male	female	XYaa	XYAa	XYAA	XXaa	XXAa	XXAA	XX*aa	XX*Aa	XX*AA	X*Yaa	X*YAa	X*YAA
XYaa	XXaa	$\frac{1}{2}$	0	0	$\frac{1}{2}$	0	0	0	0	0	0	0	0
XYaa	XXAa	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	0	0	0	0	0	0	0
XYaa	XXAA	0	$\frac{1}{2}$	0	0	$\frac{1}{2}$	0	0	0	0	0	0	0
XYaa	XX*aa	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0
XYaa	XX*Aa	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0
XYaa	XX*AA	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0
XYaa	X*Yaa	$\frac{1}{4}$	0	0	0	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0
XYaa	X*YAa	$\frac{1}{8}$	$\frac{1}{8}$	0	0	0	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0
XYaa	X*YAA	0	$\frac{1}{4}$	0	0	0	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0
XYAa	XXaa	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0	0
XYAa	XXAa	$\frac{k}{4}$	$\frac{k}{2}$	$\frac{k}{4}$	$\frac{1-k}{4}$	$\frac{1-k}{2}$	$\frac{1-k}{4}$	0	0	0	0	0	0
XYAa	XXAA	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAa	XX*aa	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYAa	XX*Aa	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$
XYAa	XX*AA	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAa	X*Yaa	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	0	0	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYAa	X*YAa	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	0	0	0	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$
XYAa	X*YAA	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	0	0	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAA	XXaa	0	*k*	0	0	*1-k*	0	0	0	0	0	0	0
XYAA	XXAa	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAA	XXAA	0	0	*k*	0	0	*1-k*	0	0	0	0	0	0
XYAA	XX*aa	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYAA	XX*Aa	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAA	XX*AA	0	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$
XYAA	X*Yaa	0	$\frac{1-k}{2}$	0	0	0	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYAA	X*YAa	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	0	0	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAA	X*YAA	0	0	$\frac{1-k}{2}$	0	0	0	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$

The dynamics of the model can be described by the following recursions:

$$
\begin{aligned}
m'_1 &= \bar{f}\left[\frac{y_1}{2}\left(x_1+\frac{x_2}{2}+\frac{x_4}{2}+\frac{x_5}{4}+\left(\frac{x_7}{2}+\frac{x_8}{4}\right)w\right)\right.\\
&\qquad\left.+\frac{y_2}{2}\left(k\left(x_1+\frac{x_2}{2}+\frac{x_4}{2}+\frac{x_5}{4}\right)+\frac{1-k}{2}\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
m'_2 &= \bar{f}\left[\frac{y_1}{2}\left(\frac{x_2}{2}+x_3+\frac{x_5}{4}+\frac{x_6}{2}+\left(\frac{x_8}{4}+\frac{x_9}{2}\right)w\right)\right.\\
&\qquad+\frac{y_2}{2}\left(k\left(x_1+x_2+x_3+\frac{x_4}{2}+\frac{x_5}{2}+\frac{x_6}{2}\right)+\frac{1-k}{2}\left(x_7+x_8+x_9\right)w\right)\\
&\qquad\left.+y_3\left(k\left(x_1+\frac{x_2}{2}+\frac{x_4}{2}+\frac{x_5}{4}\right)+\frac{1-k}{2}\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
m'_3 &= \bar{f}\left[\left(\frac{y_2}{2}+y_3\right)\left(k\left(\frac{x_2}{2}+x_3+\frac{x_5}{4}+\frac{x_6}{2}\right)+\frac{1-k}{2}\left(\frac{x_8}{2}+x_9\right)w\right)\right]\\
f'_1 &= \bar{f}\left[\left(\frac{y_1}{2}+y_2\frac{1-k}{2}\right)\left(x_1+\frac{x_2}{2}+\frac{x_4}{2}+\frac{x_5}{4}\right)\right]\\
f'_2 &= \bar{f}\left[\frac{y_1}{2}\left(\frac{x_2}{2}+x_3+\frac{x_5}{4}+\frac{x_6}{2}\right)+y_2\frac{1-k}{2}\left(x_1+x_2+x_3+\frac{x_4}{2}+\frac{x_5}{2}+\frac{x_6}{2}\right)\right.\\
&\qquad\left.+y_3(1-k)\left(x_1+\frac{x_2}{2}+\frac{x_4}{2}+\frac{x_5}{4}\right)\right]\\
f'_3 &= \bar{f}\left[\left(\frac{y_2}{2}+y_3\right)(1-k)\left(\frac{x_2}{2}+x_3+\frac{x_5}{4}+\frac{x_6}{2}\right)\right]\\
f'_4 &= \bar{f}\left[\frac{y_1}{4}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)+y_2\frac{1-k}{4}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
f'_5 &= \bar{f}\left[\frac{y_1}{4}\left(\frac{x_5}{2}+x_6+\left(\frac{x_8}{2}+x_9\right)w\right)+y_2\frac{1-k}{4}\left(x_4+x_5+x_6+\left(x_7+x_8+x_9\right)w\right)\right.\\
&\qquad\left.+y_3\frac{1-k}{2}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
f'_6 &= \bar{f}\left[\left(\frac{y_2}{2}+y_3\right)\frac{1-k}{2}\left(\frac{x_5}{2}+x_6+\left(\frac{x_8}{2}+x_9\right)w\right)\right]\\
f'_7 &= \bar{f}\left[\frac{y_1}{4}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)+y_2\frac{k}{4}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
f'_8 &= \bar{f}\left[\frac{y_1}{4}\left(\frac{x_5}{2}+x_6+\left(\frac{x_8}{2}+x_9\right)w\right)+y_2\frac{k}{4}\left(x_4+x_5+x_6+\left(x_7+x_8+x_9\right)w\right)\right.\\
&\qquad\left.+y_3\frac{k}{2}\left(x_4+\frac{x_5}{2}+\left(x_7+\frac{x_8}{2}\right)w\right)\right]\\
f'_9 &= \bar{f}\left[\left(\frac{y_2}{2}+y_3\right)\frac{k}{2}\left(\frac{x_5}{2}+x_6+\left(\frac{x_8}{2}+x_9\right)w\right)\right]
\end{aligned}
$$

Where m'_i and f'_i are the number of males of genotype i and females of genotype j at the next generation and $\bar{f}=\sum_i f_i$.

The frequencies at the next generation can be obtained as follows:

$$y'_i=\frac{m'_i}{\sum_j m'_j} \text{ and } x'_i=\frac{f'_i}{\sum_j f'_j}$$

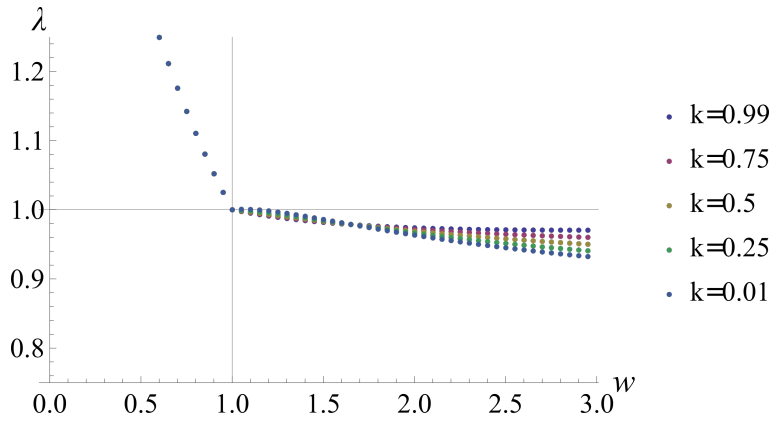
.

To determine the stability of a system with mendelian transmission of male sex chromosomes in respect to the invasion of an autosomal allele A responsible for Y-drive, we evaluate the Jacobian of the system at the equilibrium point where the allele A is absent. All males are XY

and the frequencies of XX, XX* and X*Y females are given by the eigenvector of the greatest eigenvalue of the transition matrix M_{eq1}: $\hat{y_1} = 1,\ \hat{y_2} = 0,\ \hat{y_3} = 0,\ \hat{x_1} = \frac{1}{2w-1},\ \hat{x_2} = 0,\ \hat{x_2} = 0,\ \hat{x_4} = \frac{w-1}{2w-1},\ \hat{x_5} = 0,\ \hat{x_6} = 0,\ \hat{x_7} = \frac{w-1}{2w-1},\ \hat{x_8} = 0,\ \hat{x_9} = 0.$

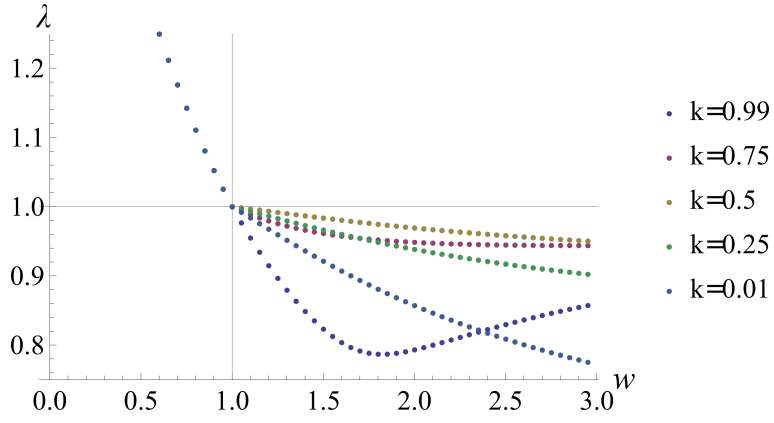
A rare A chromosome will spread if the equilibrium is locally unstable, *i.e.* if the leading eigenvalue is larger than 1, and assuming that the condition for stability of eq. 1 ($w > 1$) is satisfied.

Here the eigenvalues of the Jacobian Matrix are too complex to interpret. But stability can be investigated numerically by replacing the parameters w and k by a set of different numerical values and determining for which set of parameters the leading eigenvalue is bigger than one.



This plot shows the value of the leading eigenvalue of the Jacobian matrix for different values of w and k, ranging from 0 to 3 and 0.01 to 0.99 respectively. Assuming that $w > 1$ (condition for stability of equilibrium 1), the leading eigenvalue of the system is always smaller than one, meaning that the equilibrium is always stable *i.e.* an autosomal allele driving the transmission of males sex chromosomes could not invade in a polymorphic system with mendelian transmission of sex chromosomes.

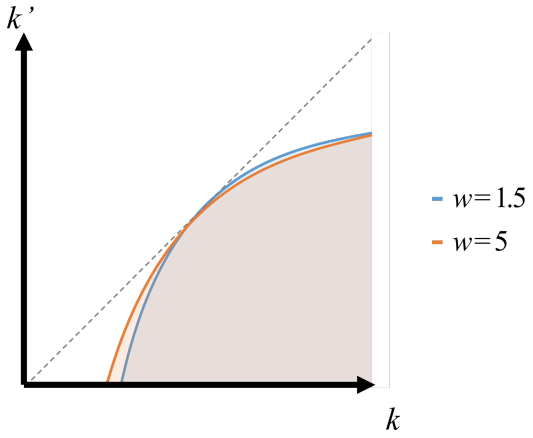
A similar model was written considering that the effect of A takes place in females, as it would if the biased transmission of male sex chromosomes was due to female cryptic choice. The results (shown below) are similar.



Step 2b': Invasion of an autosomal allele causing conditional drive of male sex chromosomes (*eq 1*). At the sex determining locus, the alleles considered are: Y, X and X*, and at the autosomal locus: a and the driving A. There are three types of males: XYaa, XYAa and XYAA and nine types of females: XXaa, XXAa, XXAA, XX*aa, XX*Aa, XX*AA, X*Yaa, X*YAa, X*YAA in number $m_1...m_3$ and $f_1...f_9$ respectively and frequencies $y_1...y_3$ and $x_1...x_9$ respectively. In all 12 types crosses, the transmission ratio of female sex chromosomes is Mendelian. Transmission of male sex chromosomes is conditioned by female genotype: transmission is mendelian in crosses with females homozygous for the autosomal a allele, and biased in crosses with females heterozygous or homozygous for the autosomal A allele, the strength and direction of bias depends on female genotype at the sex-determining locus: in crosses involving XX or XX* females, male Y chromosome has a transmission ratio of k, in crosses involving X*Y females (see table on opposite page), of $k*$. Sex reversed females (X*Yaa, X*YAa and X*YAA) have a fertility w.

To determine the stability of a system with Mendelian transmission of male sex chromosomes in respect to the invasion of an autosomal allele causing conditional drive of male sex chromosomes, in a similar way to step 2b, the recursion equations were written and the Jacobian matrix was evaluated at the *eq 1*. A rare A allele will spread if the leading eigenvalue of the Jacobian is larger than 1, and assuming that the condition for stability of *eq 1* ($w > 1$) is satisfied.

Once again, the eigenvalues of the Jacobian are too complex to interpret. Stability was investigated numerically by replacing the parameters w, k and $k*$ by a set of different values and determining for which set of parameters the leading eigenvalue is bigger than one (see figure below).



This figure shows the space of parameters (k and $k*$) allowing the invasion of a rare A mutant causing conditional TDMSC, for two values of w (> 1) (shaded zone). For the mutant A to invade, $k*$ must be smaller than k, but invasion is not possible for high values of $k*$ (around 0.75, and even if k is larger) or low values of k (around 0.3, and even if k* is smaller). w has overall little impact.

crosses		offspring genotypes											
male	female	XYaa	XYAa	XYAA	XXaa	XXAa	XXAA	XX*aa	XX*Aa	XX*AA	X*Yaa	X*YAa	X*YAA
XYaa	XXaa	$\frac{1}{2}$	0	0	$\frac{1}{2}$	0	0	0	0	0	0	0	0
XYaa	XXAa	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0	0
XYaa	XXAA	0	k	0	0	*1-k*	0	0	0	0	0	0	0
XYaa	XX*aa	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0
XYaa	XX*Aa	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYaa	XX*AA	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYaa	X*Yaa	$\frac{1}{4}$	0	0	0	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0
XYaa	X*YAa	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$	0
XYaa	X*YAA	0	$\frac{1-k^*}{2}$	0	0	0	0	0	$\frac{1-k^*}{2}$	0	0	$\frac{k^*}{4}$	0
XYAa	XXaa	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	0	0	0	0	0	0	0
XYAa	XXAa	$\frac{k}{4}$	$\frac{k}{2}$	$\frac{k}{4}$	$\frac{1-k}{4}$	$\frac{1-k}{2}$	$\frac{1-k}{4}$	0	0	0	0	0	0
XYAa	XXAA	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAa	XX*aa	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0
XYAa	XX*Aa	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$
XYAa	XX*AA	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAa	X*Yaa	$\frac{1}{8}$	$\frac{1}{8}$	0	0	0	0	$\frac{1}{8}$	$\frac{1}{8}$	0	$\frac{1}{8}$	$\frac{1}{8}$	0
XYAa	X*YAa	$\frac{1-k^*}{8}$	$\frac{1-k^*}{4}$	$\frac{1-k^*}{8}$	0	0	0	$\frac{1-k^*}{8}$	$\frac{1-k^*}{4}$	$\frac{1-k^*}{8}$	$\frac{k^*}{8}$	$\frac{k^*}{4}$	$\frac{k^*}{8}$
XYAa	X*YAA	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$
XYAA	XXaa	0	$\frac{1}{2}$	0	0	$\frac{1}{2}$	0	0	0	0	0	0	0
XYAA	XXAa	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAA	XXAA	0	0	k	0	0	*1-k*	0	0	0	0	0	0
XYAA	XX*aa	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0
XYAA	XX*Aa	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAA	XX*AA	0	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$
XYAA	X*Yaa	0	$\frac{1}{4}$	0	0	0	0	0	$\frac{1}{4}$	0	0	$\frac{1}{4}$	0
XYAA	X*YAa	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$
XYAA	X*YAA	0	0	$\frac{1-k^*}{2}$	0	0	0	0	0	$\frac{1-k^*}{2}$	0	0	$\frac{k^*}{2}$

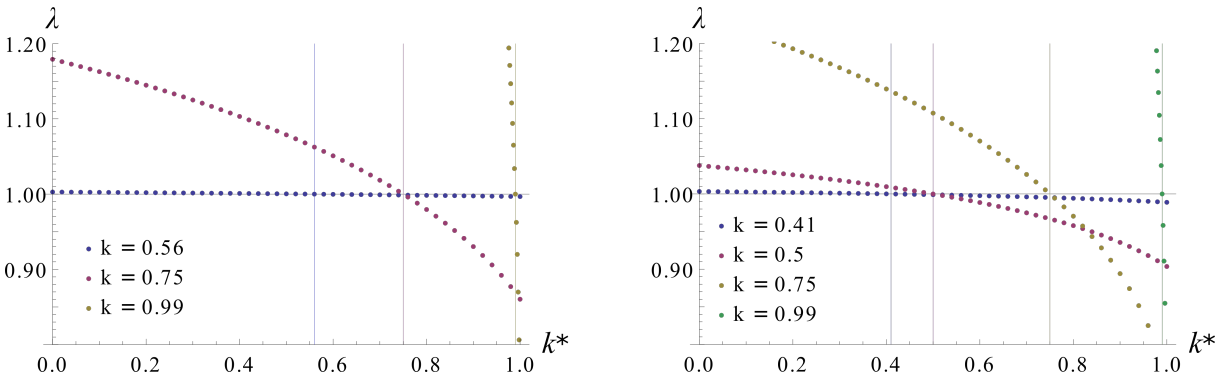
Step 3: Invasion of modifier of TDMSC specific to XY x X*Y crosses in a system with an existing unconditional TDMSC (*eq 2*). At the sex determining locus, the alleles considered are: Y, X and X*, and at the autosomal locus: a and the driving A. There are three types of males: XYaa, XYAa and XYAA and nine types of females: XXaa, XXAa, XXAA, XX*aa, XX*Aa, XX*AA, X*Yaa, X*YAa, X*YAA in number $m_1...m_3$ and $f_1...f_9$ respectively and frequencies $y_1...y_3$ and $x_1...x_9$ respectively. In all 12 types crosses, the transmission ratio of female sex chromosomes is Mendelian. In crosses involving X*YAa and X*YAA females, male Y chromosome has a transmission ratio of k^* and in all other crosses transmission ratio is of k (see table below). Sex reversed females (X*Yaa, X*YAa and X*YAA) have a fertility w.

crosses		offspring genotypes											
male	female	XYaa	XYAa	XYAA	XXaa	XXAa	XXAA	XX*aa	XX*Aa	XX*AA	X*Yaa	X*YAa	X*YAA
XYaa	XXaa	k	0	0	*1-k*	0	0	0	0	0	0	0	0
XYaa	XXAa	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0	0
XYaa	XXAA	0	k	0	0	*1-k*	0	0	0	0	0	0	0
XYaa	XX*aa	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0	0
XYaa	XX*Aa	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYaa	XX*AA	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYaa	X*Yaa	$\frac{1-k}{2}$	0	0	0	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0	0
XYaa	X*YAa	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$	0
XYaa	X*YAA	0	$\frac{1-k^*}{2}$	0	0	0	0	0	$\frac{1-k^*}{2}$	0	0	$\frac{k^*}{4}$	0
XYAa	XXaa	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0	0
XYAa	XXAa	$\frac{k}{4}$	$\frac{k}{2}$	$\frac{k}{4}$	$\frac{1-k}{4}$	$\frac{1-k}{2}$	$\frac{1-k}{4}$	0	0	0	0	0	0
XYAa	XXAA	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAa	XX*aa	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYAa	XX*Aa	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{1-k}{8}$	$\frac{1-k}{4}$	$\frac{1-k}{8}$	$\frac{k}{8}$	$\frac{k}{4}$	$\frac{k}{8}$
XYAa	XX*AA	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAa	X*Yaa	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	0	0	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$	0
XYAa	X*YAa	$\frac{1-k^*}{8}$	$\frac{1-k^*}{4}$	$\frac{1-k^*}{8}$	0	0	0	$\frac{1-k^*}{8}$	$\frac{1-k^*}{4}$	$\frac{1-k^*}{8}$	$\frac{k^*}{8}$	$\frac{k^*}{4}$	$\frac{k^*}{8}$
XYAa	X*YAA	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$
XYAA	XXaa	0	k	0	0	*1-k*	0	0	0	0	0	0	0
XYAA	XXAa	0	$\frac{k}{2}$	$\frac{k}{2}$	0	$\frac{1-k}{2}$	$\frac{1-k}{2}$	0	0	0	0	0	0
XYAA	XXAA	0	0	k	0	0	*1-k*	0	0	0	0	0	0
XYAA	XX*aa	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYAA	XX*Aa	0	$\frac{k}{4}$	$\frac{k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{1-k}{4}$	$\frac{1-k}{4}$	0	$\frac{k}{4}$	$\frac{k}{4}$
XYAA	XX*AA	0	0	$\frac{k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$
XYAA	X*Yaa	0	$\frac{1-k}{2}$	0	0	0	0	0	$\frac{1-k}{2}$	0	0	$\frac{k}{2}$	0
XYAA	X*YAa	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	0	0	0	$\frac{1-k^*}{4}$	$\frac{1-k^*}{4}$	0	$\frac{k^*}{4}$	$\frac{k^*}{4}$
XYAA	X*YAA	0	0	$\frac{1-k^*}{2}$	0	0	0	0	0	$\frac{1-k^*}{2}$	0	0	$\frac{k^*}{2}$

To determine the stability of *eq 2* in respect to the invasion of an distorter specific to X*Y females on an autosome, in a similar way to step 2b, the recursion equations were written and the Jacobian matrix was evaluated at this equilibrium. All males are XY and the frequencies of XX, XX* and X*Y females are given by the eigenvector of the greatest eigenvalue of the transition matrix M_{eq2}: $\hat{y_1} = 1,\ \hat{y_2} = 0,\ \hat{y_3} = 0,\ \hat{x_1} = \frac{(1-k)^2}{k(k+w-1)},\ \hat{x_2} = 0,\ \hat{x_3} = 0,\ \hat{x_4} = \frac{(1-k)(k+kw-1)}{k(k+w-1)},\ \hat{x_5} = 0,\ \hat{x_6} = 0, \hat{x_7} = \frac{k+kw-1}{k+w-1},\ \hat{x_8} = 0,\ \hat{x_9} = 0.$

A rare A allele will spread if the leading eigenvalue of the Jacobian is larger than 1, and assuming that the condition for stability of equilibrium *eq 2* ($w > \frac{1-k}{k}$) is satisfied.

Once again, the eigenvalues of the Jacobian are too complex to interpret. Stability was investigated numerically by replacing the parameters w, k and k^* by a set of different numerical values and determining for which set of parameters the leading eigenvalue is bigger than one (see figure below).



Assuming that $w > \frac{1-k}{k}$, the leading eigenvalue is greater than one for $k^* < k$, for values of w smaller and greater than 1 (here 0.8 and 1.5). So an autosomal allele specifically biasing the transmission ratio of male sex chromosomes in a X*Y context will invade provided that it increases the transmission of its partner's X chromosome.

Invasion of a mutant X*

The study of the invasion of mutations on the X* is different as it does not involve non-linear models (no males harbour the mutation so models are linear). Invasion conditions can be obtained through the study of the transition matrices (see Appendix A).

Step 2a: Invasion of an X* in a classic sex determination system with Y chromosome drive.

The condition for invasion corresponds to the condition of stability of equilibrium 2: $w > \frac{1-k}{k}$.

Step 2a': Invasion of an X* modifying male Y chromosome drive in XY x X*Y crosses, in a classic sex determination system with Y chromosome drive.

The condition for invasion corresponds to the condition of stability of equilibrium 3: $w > \frac{1-k}{k} * \frac{1-k}{\frac{1}{2}-k^*(1-\frac{1}{2k})}$.

Step 3: Invasion of an X* modifying male Y chromosome drive in XY x X*Y crosses, in a polygenic system system with an unconditional TDMSC.

There is one type of males, and five types of females: XX, XX*, XX*^{d}, X*Y, X*^{d}Y in number ($f_1 ... f_5$)

The dynamics of the model is given by the recursions:

$$\begin{bmatrix} f_1 \\ \vdots \\ f_5 \end{bmatrix}_{(t+1)} = M \begin{bmatrix} f_1 \\ \vdots \\ f_5 \end{bmatrix}_{(t)}$$

With the transition matrix:

$$M = \begin{bmatrix} 1-k & \frac{1-k}{2} & \frac{1-k}{2} & 0 & 0 \\ 0 & \frac{1-k}{2} & 0 & w\frac{1-k}{2} & 0 \\ 0 & 0 & \frac{1-k}{2} & 0 & w\frac{1-k^*}{2} \\ 0 & \frac{k}{2} & 0 & w\frac{k}{2} & 0 \\ 0 & 0 & \frac{k}{2} & 0 & w\frac{k^*}{2} \end{bmatrix}$$

m_{ij} elements describe the number of females of genotype i in the progeny of a female of genotype j.

The three leading eigenvalues are: $\lambda_1 = 1-k$, $\lambda_1 = \frac{1}{2}(1+k(w-1))$ and $\lambda_3 = \frac{1}{4}(1-k+k^*w+\sqrt{k^2-2k(1+(k^*-2)w)+(k^*-1)^2}$.

According to the eigenvectors, the X*^{d} can only invade if λ_3 is the leading eigenvalue.
$\lambda_3 > \lambda_2$ if $w > \frac{1-k}{k} * \frac{1-k}{\frac{1}{2}-k^*(1-\frac{1}{2k})}$ (condition for stability of *eq 3* (see Appendix A).
$\lambda_3 > \lambda_1$ if (i) $k^* > k$ (when $w > 1$); (ii) $k^* < k$ (when $\frac{1-k}{k} < w < 1$).

The condition for invasion of a X*^{d} depends on X*Y females fecundity: if it is superior to that of XX and XX* females ($w > 1$), the original X* will be replaced provided that the new one increases the strength of Y drive ($k* > k$), but if X*Y females have a lower fitness ($w < 1$), then the X*^{d} will invade provided it decreases Y drive ($k^* < k$).

Supplementary material of manuscript 4

Resident intruder test

The effect of genotype on latency to first attack and number of aggressions was analysed using generalized mixed models (*glmer* function in R), with respectively geometric and gamma distribution. *Male ID* was set as a random effect, and *female : male mass ratio* (♀/♂ *mass ratio*) and *male trial number* (one to three) were set as fix covariates. Model simplification was made using Likelihood ratio tests (LRT). Post-hoc comparisons were made using Tukey's HSD tests. Variables with significant effect are highlighted in bold.

Latency to first attack (sec)

XX (mean+/-SD)	XX*	X*Y
192.04+/-172.14	119.09+/-93.41	68.9+/-47.56

Model (fixed effects): ~ Genotype * Mass-ratio + Trial	Variable tested	Statistic
~ Genotype + Mass-ratio + Trial	Genotype : ♀/♂ Mass-ratio interaction	X_2=0.81, p=0.67
~ Genotype + Trial	♀/♂ Mass ratio	X_1=0.2, p=0.66
~ Genotype	♂ trial number	X_1=1.75, p=0.19
~ 1	**Genotype**	**X^2_2=7.0, p=0.029**

Number of aggressions (attacks and chases)

XX (mean+/-SD)	XX*	X*Y
4.36+/-8.07	4.33+/-4.96	13.63+/-13.03

Model (fixed effects): ~ Genotype * Mass-ratio + Trial	Variable tested	Statistic
~ Genotype + Mass-ratio + Trial	Genotype : ♀/♂ Mass-ratio interaction	X_2=1.67, p=0.43
~ Genotype + Trial	♀/♂ Mass ratio	X_1=0.02, p=0.90
~ Genotype	♂ trial number	X_1=0.02, p=0.89
~ 1	**Genotype**	**X^2_2=11.00, p=0.004**

	Tukey's HSD tests		
	XX vs. XX*	**XX vs. X*Y**	**XX* vs. X*Y**
Latency to first attack	p=0.45	p=0.27	**p=0.037**
Number of aggressions	p=0.93	**p=0.01**	**p=0.03**

Dark Light Box and Open Field

The effect of sex chromosomes (X* and Y) on variables measured in the Dark-Light box and the Open Field was assessed using MANOVAs. Three covariates were used: the *age* of individuals at the time of the trial; the *group* in which they were tested (from 1 to 12, animals were tested four by four on the same day); the *location* of the individual (upper/lower –right/left). Statistical inference was made using LRT with Fisher's exact test. A significant effect of Y chromosome was found in the Dark light box. Univariate ANOVAs were used to assess which variables were affected. Significant values (p-value<0.05) are highlighted in bold.

Dark light Box

MANOVA

Model: ~ Y * X* + Group + Age + Position	Variable tested	Statistics
~ Y + X* + Group + Age + Position	Y : X* interaction	Pillai=0.18, $F_{1,37}$=2.49 p=0.08
~ Y + Group + Age + Position	X* chromosome	Pillai=0.02, $F_{1,38}$=0.28 p=0.66
~ Y + Group + Age	Position	Pillai=0.04, $F_{1,39}$=0.53 p=0.66
~ Y + Age	Group	Pillai=0.09, $F_{1,40}$=1.30 p=0.29
~ Y	**Age**	**Pillai=0.20, $F_{1,41}$=3.34, p=0.03**
~ 1	**Y chromosome**	**Pillai=0.20, $F_{1,41}$=3.30, p=0.03**

Univariate ANOVAS

Time spent in the light box (sec)

XX (mean+/-SD)	XX*	X*Y	XY
117.66+/-53.79 (sec)	87.80+/-61.09	115.02+/-58.56	105.41+/-69.96

Model: ~ Y * X* + Group + Age + Position	Variable tested	Statistic
~ Y + X* + Group + Age + Position	Y : X* interaction	$F_{1,36}$=2.61 p=0.11
~ Y + X* + Group + Position	Age	$F_{1,37}$=1e-4, p=0.99
~ X* + Group + Position	Y chromosome	$F_{1,38}$=1e-3, p=0.97
~ Group + Position	X* chromosome	$F_{1,39}$=0.11, p=0.89
~ Position	Group	$F_{1,40}$=0.50, p=0.48
~ 1	Position	$F_{3,41}$=1.31, p=0.28

Distance covered (centimetres)

One female removed from dataset (X*Y, distance over 9000 cm)

XX (mean+/-SD)	XX*	X*Y	XY
2376.29+/-751.39	2380.67+/-976.63	2958.56+/-1172.93	2871.01+/-975.09

Model: ~ Y * X* + Group + Age + Position	Variable tested	Statistic
~ Y + X* + Group + Age + Position	Y : X* interaction	$F_{1,36}$=3.01 p=0.09
~ Y + X* + Group + Age	Position	$F_{3,37}$=0.14, p=0.93
~ Y + X* + Age	Group	$F_{1,40}$=2.85, p=0.10
~ Y + Age	X*	$F_{1,41}$=0.073, p=0.79
~ Age	**Y**	**$F_{1,43}$=4.64, p=0.04**
~ Y	**Age**	**$F_{1,43}$=3.89, p=0.054**

Time spend in a static posture in the first 120 seconds (sec)

XX (mean+/-SD)	XX*	X*Y	XY
44.57+/-21.87	44.97+/-22.77	26.72+/-15.77	23.64+/-12.57

Model: ~ Y * X* + Group + Age + Position	Variable tested	Statistic
~ Y + X* + Group + Age + Position	Y : X* interaction	$F_{1,37}$=0.76 p=0.39
~ Y + Group + Age + Position	X*	$F_{1,38}$=6e-3, p=0.94
~ Y + Group + Position	Age	$F_{1,39}$=0.14, p=0.71
~ Y + Position	Group	$F_{1,40}$=1.79, p=0.19
~ Y	**Position**	**$F_{3,41}$=3.00, p=0.041**
~ Position	**Y**	**$F_{1,41}$=10.97, p=0.002**

Open field

MANOVA

Model: ~ Y * X* + Group + Age + Position	Variable tested	Statistics
~ Y + X* + Group + Age + Position	Y : X* interaction	Pillai=0.03, $F_{1,37}$=0.38 p=0.77
~ Y + Group + Age + Position	X* chromosome	Pillai=2e-3, $F_{1,38}$=0.04 p=0.99
~ Y + Group + Position	Age	Pillai=0.03, $F_{1,39}$=0.39 p=0.76
~ Y + Group	Position	Pillai=0.20, $F_{3,40}$=0.95 p=0.48
~ Y	Group	Pillai=0.20, $F_{1,41}$=0.24, p=0.26
~ 1	Y chromosome	Pillai=0.13, $F_{1,43}$=2.19, p=0.10

Bibliographie

Ahmed, S., J. M. Cock, E. Pessia, R. Luthringer, A. Cormier, M. Robuchon, L. Sterck, A. F. Peters, S. M. Dittami, E. Corre, M. Valero, J.-M. Aury, D. Roze, Y. Van de Peer, J. Bothwell, G. a B. Marais, and S. M. Coelho. 2014. A haploid system of sex determination in the brown alga Ectocarpus sp. Curr. Biol. 24:1945–57. Elsevier Ltd.

Arnold, A. 2012. The end of gonad-centric sex determination in mammals. Trends Genet. 28:55–61.

Bachtrog, D. 2006. A dynamic view of sex chromosome evolution. Curr. Opin. Genet. Dev. 16:578–585.

Bachtrog, D. 2014. Signs of Genomic Battles in Mouse Sex Chromosomes. Cell 159:716–718.

Bachtrog, D. 2013. Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. 14:113–24. Nature Publishing Group.

Bachtrog, D., E. Hom, K. M. Wong, X. Maside, and P. de Jong. 2008. Genomic degradation of a young Y chromosome in *Drosophila miranda*. Genome Biol. 9:R30.

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T.-L. Ashman, M. W. Hahn, J. Kitano, I. Mayrose, R. Ming, N. Perrin, L. Ross, N. Valenzuela, and J. C. Vamosi. 2014. Sex Determination: Why So Many Ways of Doing It? PLoS Biol. 12:e1001899.

Baird, N. a, P. D. Etter, T. S. Atwood, M. C. Currey, A. L. Shiver, Z. a Lewis, E. U. Selker, W. a Cresko, and E. a Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:e3376.

Baroiller, J. F., H. D'Cotta, E. Bezault, S. Wessels, and G. Hoerstgen-Schwark. 2009. Tilapia sex determination: Where temperature and genetics meet. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 153:30–8. Elsevier Inc.

Bellott, D. W., J. F. Hughes, H. Skaletsky, L. G. Brown, T. Pyntikova, T.-J. Cho, N. Koutseva, S. Zaghlul, T. Graves, S. Rock, C. Kremitzki, R. S. Fulton, S. Dugan, Y. Ding, D. Morton, Z. Khan, L. Lewis, C. Buhay, Q. Wang, J. Watt, M. Holder, S. Lee, L. Nazareth, J. Alföldi, S. Rozen, D. M. Muzny, W. C. Warren, R. a Gibbs, R. K. Wilson, and D. C. Page. 2014. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. Nature 508:494–9.

Benenson, I. E. 1983. On the maintenance of the unique system of sex determination in Lemmings. Oikos 41:211–218.

Bergero, R., and D. Charlesworth. 2009. The evolution of restricted recombination in sex chromosomes. Trends Ecol. Evol. 24:94–102.

Beukeboom, L. W., and N. Perrin. 2014. The Evolution of Sex Determination. Oxford University Press.

Bianchi, N. O. 2002. *Akodon* sex reversed females: the never ending story. Cytogenet. Genome Res. 96:497–501.

Bininda-Emonds, O. R. P., M. Cardillo, K. E. Jones, R. D. E. MacPhee, R. M. D. Beck, R. Grenyer, S. a Price, R. a Vos, J. L. Gittleman, and A. Purvis. 2007. The delayed rise of present-day mammals. Nature 446:507–12.

Blaser, O., C. Grossen, S. Neuenschwander, and N. Perrin. 2012. Sex-Chromosome Turnovers Induced By Deleterious Mutation Load. Evolution (N. Y). 67:635–645.

Bortolato, M., S. C. Godar, S. Davarian, K. Chen, and J. C. Shih. 2009. Behavioral disinhibition and reduced anxiety-like behaviors in monoamine oxidase B-deficient mice. Neuropsychopharmacology 34:2746–2757.

Britton-Davidian, J., T. J. Robinson, and F. Veyrunes. 2012. Systematics and evolution of the African pygmy mice, subgenus Nannomys: a review. Acta Oecol. 42:41–49. Elsevier Masson SAS.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park. CA.

Bull, J. J., and E. L. Charnov. 1977. Changes in the heterogametic mechanism of sex determination. Heredity (Edinb). 39:1–14.

Bull, J. J., and E. L. Charnov. 1988. How fundamental are Fisharian sex ratios? Oxford Surv. Evol. Biol. 5:96–135.

Burt, A., and R. Trivers. 2006. Genes in conflict: the biology of selfish genetic elements. Cambridge.

Carruth, L. L., I. Reisert, and A. P. Arnold. 2002. Sex chromosome genes directly affect brain sexual differentiation. Nat. Neurosci. 5:933–34.

Cases, O., I. Seif, J. Grimsby, and P. Gaspar. 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science (80-.). 268:1763–6.

Catzeflis, F., and C. Denys. 1992. The African Nannomys (Muridae): an early offshoot from the Mus lineage. Evidence from scnDNA hybridization experiments and compared morphology. Isr. J Zool 38:117–124.

Charlesworth, B. 1991. The Evoluton of Sex Chromosomes. Science (80-.). 251:1–4.

Charlesworth, B., and D. Charlesworth. 2000. The degeneration of Y chromosomes. Phil. Trans. R. Soc. Lond. B 355:1563–72.

Charnov, E. L. 1982. The Theory of Sex Allocation. Princenton.

Cocquet, J., P. J. I. Ellis, S. K. Mahadevaiah, N. a Affara, D. Vaiman, and P. S. Burgoyne. 2012. A genetic basis for a postmeiotic x versus y chromosome intragenomic conflict in the mouse. PLoS Genet. 8:e1002900.

Cordaux, R., D. Bouchon, and P. Grève. 2011. The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends Genet. 27:332–41.

Cox, K. H., P. J. Bonthuis, and E. F. Rissman. 2014. Mouse model systems to study sex chromosome genes and behavior: Relevance to humans. Front. Neuroendocr. 35:405–19. Elsevier Inc.

Devlin, R. H., and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191–364.

Dewing, P., C. W. K. Chiang, K. Sinchak, H. Sim, P.-O. Fernagut, S. Kelly, M.-F. Chesselet, P. E. Micevych, K. H. Albrecht, V. R. Harley, and E. Vilain. 2006. Direct regulation of adult brain function by the male-specific factor SRY. Curr. Biol. 16:415–20.

Disteche, C. M. 2012. Dosage compensation of the sex chromosomes. Annu. Rev. Genet. 46:537–60.

Dubendorfer, A., D. Hilfiker-Kleiner, and R. Nothiger. 1992. Sex determination mechanisms in dipteran insects: the case of Musca domestica. Dev. Biol. 3:349–356.

Eggers, S., and A. Sinclair. 2012. Mammalian sex determination—insights from humans and mice. Chromosom. Res. 20:215–38.

Espinosa, M. B., and A. D. Vitullo. 1996. Offspring sex-ratio and reproductive performance in heterogametic females of the South American field mouse *Akodon azarae*. Hereditas 124:57–62.

Ezaz, T., A. E. Quinn, I. Miura, S. D. Sarre, A. Georges, and J. a Marshall Graves. 2005. The dragon lizard Pogona vitticeps has ZZ/ZW micro-sex chromosomes. Chromosome Res. 13:763–76.

Fichet-Calvet, E., L. Audenaert, P. Barrière, and E. Verheyen. 2009. Diversity, dynamics and reproduction in a community of small mammals in Upper Guinea, with emphasis on pygmy mice ecology. Afr. J. Ecol. 48:600–614.

Fisher, R. A. 1930. The genetical theory of natural selection.

Fredga, K. 1994. Bizarre mammalian sex-determining mechanisms. *in* R. V Short and E. Balaban, eds. The differences between the sexes. Cambridge University Press.

Fredga, K., and M. Bulmer. 1988. Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. Phil. Trans. R. Soc. Lond. B 322:83–95.

Fredga, K., A. Gropp, H. Winking, and F. Frank. 1976. Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*. Nature 261:225–227.

Gamble, T., J. Coryell, T. Ezaz, J. Lynch, D. P. Scantlebury, and D. Zarkower. 2015. Restriction Site-Associated DNA Sequencing (RAD-seq) Reveals an Extraordinary Number of Transitions among Gecko Sex-Determining Systems. Mol. Biol. Evol. 32:1296–309.

Gamble, T., and D. Zarkower. 2014. Identification of sex-specific molecular markers using restriction site-associated DNA sequencing. Mol. Ecol. Resour. 14:902–13.

Gardner, A., and L. Ross. 2014. Mating ecology explains patterns of genome elimination. Ecol. Lett. 17:1602–1612.

Gileva, E. A. 1987. Meiotic drive in the sex chromosome system of the varying lemming , Dicrostonyx torquatus Pall. (Rodentia, Microtinae). Heredity (Edinb). 59:383–389.

Gileva, E. A., I. E. Benenson, L. A. Konopistseva, V. F. Puchkov, and I. A. Makaranets. 1982. XO Females in the Varying Lemming , *Dicrostonyx torquatus* : Reproductive Performance and its Evolutionary Significance. Evolution (N. Y). 36:601–609.

Gross, M. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol. Evol. 11:92–7.

Hamilton, W. D. 1967. Extraordinary Sex Ratios. Science (80-.). 156:477–488.

Happold, D. C. D. 2013. Mammals of Africa. volume 3: Rodentia. University of California Press, Berkeley.

Holleley, C. E., D. O'Meally, S. D. Sarre, J. a. Marshall Graves, T. Ezaz, K. Matsubara, B. Azad, X. Zhang, and A. Georges. 2015. Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. Nature 523:79–82.

Honda, T., and M. Itoh. 1977. An unusual sex chromosome constitution found in the Amami spinous country-rat, Tokudaia osimensis osimensis. Jpn J. Genet. 52:247–9.

Hurst, L. D., and J. P. Randerson. 1999. An eXceptional chromosome. Trends Genet. 15:383–5.

Jiménez, R., F. J. Barrionuevo, and M. Burgos. 2013. Natural Exceptions to Normal Gonad Development in Mammals. Sex. Dev. 7:147–162.

Jotterand-Bellomo, M. 1988. Chromosome analysis of five specimens of Mus bufo-triton (Muridae) from Burundi (Africa): three cytogenetic entities, a special type of chromosomal sex determination, taxonomy, and phylogeny. Cytogenet. Genome Reasearch 48:88:91.

Just, W., W. Rau, W. Vogen, M. Akhverdian, K. Fredga, J. A. M. Marshall Graves, and E. Lyapunova. 1995. Absence of Sry in species of the vole Ellobius. Nat. Genet. 11:117–18.

Kashimada, K., and P. Koopman. 2010. Sry: the master switch in mammalian sex determination. Development 137:3921–30.

Khil, P. P., N. a Smirnova, P. J. Romanienko, and R. D. Camerini-Otero. 2004. The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. Nat. Genet. 36:642–646.

Kozielska, M., F. J. Weissing, L. W. Beukeboom, and I. Pen. 2010. Segregation distortion and the evolution of sex-determining mechanisms. Heredity (Edinb). 104:100–112.

Kuroiwa, A., Y. Ishiguchi, F. Yamada, A. Shintaro, and Y. Matsuda. 2010. The process of a Y-loss event in an XO/XO mammal, the Ryukyu spiny rat. Chromosoma 119:519–526.

Lahn, B. T., and D. C. Page. 1999. Four Evolutionary Strata on the Human X Chromosome. Science (80-.). 286:964–967.

Mank, J. 2009. The W, X, Y and Z of sex-chromosome dosage compensation. Trends Genet. 25:226–233.

Mank, J. E. 2013. Sex chromosome dosage compensation: definitely not for everyone. Trends Genet. 29:677–83. Elsevier Ltd.

Mank, J. E. 2012. Small but mighty: the evolutionary dynamics of W and Y sex chromosomes. Chromosom. Res. 20:21–33.

Mank, J. E., and J. C. Avise. 2009. Evolutionary diversity and turn-over of sex determination in teleost fishes. Sex Dev. 3:60–7.

Marshall Graves, J. a. 2008. Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. Annu. Rev. Genet. 42:565–86.

Marshall Graves, J. A. M. 2006. Sex chromosome specialization and degeneration in mammals. Cell 124:901–14.

Matsubara, K., H. Tarui, M. Toriba, K. Yamada, C. Nishida-Umehara, K. Agata, and Y. Matsuda. 2006. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc. Natl. Acad. Sci. U. S. A. 103:18190–5.

Matthey, R. 1965. Le problème de la détermination du sexe chez *Acomys selousi* de Winton. (Rodentia-Murinae). Rev. Suisse Zool. 72:119–144.

Maynard Smith, J., and N. C. Stenseth. 1978. On The Evolutionary Stability Of The Female-Biased Sex-Ratio In The Wood Lemming (*Myopus schisticolor*): The Effect Of Inbreeding. Heredity (Edinb). 41:205–214.

Monadjem, A. 1999. Population dynamics of *Mus minutoides* and *Steatomys pratensis* (Muridae : Rodentia) in a subtropical grassland in Swaziland. Afr. J. Ecol. 37:202–210.

Moore, E. C., and R. B. Roberts. 2013. Polygenic sex determination. Curr. Biol. 23:R510–2. Elsevier.

Mueller, J. L., S. K. Mahadevaiah, P. J. Park, P. E. Warburton, D. C. Page, and J. M. a Turner. 2008. The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat. Genet. 40:794–9.

Mueller, J. L., H. Skaletsky, L. G. Brown, S. Zaghlul, S. Rock, T. Graves, K. Auger, W. C. Warren, R. K. Wilson, and D. C. Page. 2013. Independent specialization of the human and mouse X chromosomes for the male germ line. Nat. Genet. 45:1083–7. Nature Publishing Group.

Musser, G. G., and M. D. Carleton. 2005. Family Muridae. Pp. 501–755 *in* Mammal species of the world. A taxonomic and geographique reference. Washington and London : Smithsonian Institution Press.

Nam, K., and H. Ellegren. 2008. The chicken (Gallus gallus) Z chromosome contains at least three nonlinear evolutionary strata. Genetics 180:1131–6.

Nanda, I., U. Hornung, M. Kondo, M. Schmid, and M. Schartl. 2003. Common Spontaneous Sex-Reversed XX males of the Medaka Oryzias latipes. 251:245–251.

Ohno, S. 1967. Sex Chromosomes and Sex-linked Genes. Springer-Verlag.

Ohno, S., J. Jainchill, and C. Stenius. 1963. The creeping vole (Microtus oregoni) as a gonosomic mosaic. I. The 0Y/XY constitution of the male. Cytogenetics 2:232–239.

Pokorná, M., and L. Kratochvíl. 2009. Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? Zool. J. Linn. Soc.-Lond. 156:168–83.

Quinn, A., A. Georges, S. Sarre, and F. Guarino. 2007. Temperature sex reversal implies sex gene dosage in a reptile. Science (80-.). 316:2007.

Quinn, A., and P. Koopman. 2012. The molecular genetics of sex determination and sex reversal in mammals. Semin. Reprod. Med. 30:351–63.

Rice, W. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution (N. Y). 38:735–742.

Rice, W. R., and N. May. 2007. On the Instability of Polygenic Sex Determination : The Effect of Sex- Specific Selection. 40:633–639.

Roco, Á. S., A. W. Olmstead, S. J. Degitz, T. Amano, L. B. Zimmerman, and M. Bullejos. 2015. Coexistence of Y, W, and Z sex chromosomes in *Xenopus tropicalis*. Proc. Natl. Acad. Sci. 201505291.

Sahara, K., A. Yoshido, and W. Traut. 2012. Sex chromosome evolution in moths and butterflies. Chromosome Res. 20:83–94.

Saifi, G. M., and H. S. Chandra. 1999. An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc. Biol. Sci. 266:203–209.

Sander van Doorn, G. 2013. Evolutionary Transitions between Sex-Determining Mechanisms: A Review of Theory. Sex. Dev., doi: 10.1159/000357023.

Sander van Doorn, G. 2014. Patterns and mechanisms of evolutionary transitions between genetic sex-determining systems. Cold Spring Harb. Perspect. Biol. 6.

Sarre, S. D., A. Georges, and A. Quinn. 2004. The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. Bioessays 26:639–45.

Sekido, R. 2014. The potential role of SRY in epigenetic gene regulation during brain sexual differentiation in Mammals. Pp. 135–65 *in* Epigenetic Shaping of Sociosexual Interactions: From Plants to Humans. Elsevier.

Ser, J. R., R. B. Roberts, and T. D. Kocher. 2011. Multiple interacting loci control sex determination in lake malawi cichlid fish. 64:486–501.

Shuker, D. M., and S. A. West. 2004. Information constraints and the precision of adaptation: Sex ratio manipulation in wasps. Proc. Natl. Acad. Sci. 101:10363–10367.

Sinclair, A. H., P. Berta, M. S. Palmer, J. R. Hawkins, B. L. Griffiths, M. J. Smith, J. W. Foster, A. M. Frischauf, R. Lovell-Badge, and P. N. Goodfellow. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240–244.

Skinner, J. D., and C. T. Chimimba. 2005. The Mammals of the Southern African Subregion. 3rd ed. Cambridge University Press, New York.

Skuse, D. H. 2006. Sexual dimorphism in cognition and behaviour: the role of X-linked genes. Eur. J. Endocrinol. 155:S99–S106.

Skuse, D. H. 2005. X-linked genes and mental functioning. Hum. Mol. Genet. 14 Spec No:R27–32.

Soh, Y. Q. S., J. Alföldi, T. Pyntikova, L. G. Brown, and T. Graves. 2014. Sequencing the Mouse Y Chromosome Reveals Convergent Gene Acquisition and Amplification on Both Sex Chromosomes. Cell.

Soullier, S., C. Hanni, F. Catzeflis, P. Berta, and V. Laudet. 1998. Male sex determination in the spiny rat *Tokudaia osimensis* (Rodentia: Muridae) is not *Sry* dependent. Mamm. Genome 9:590–592.

Tree Of Sex Consortium, T., T.-L. Ashman, D. Bachtrog, H. Blackmon, E. E. Goldberg, M. W. Hahn, M. Kirkpatrick, J. Kitano, J. E. Mank, I. Mayrose, R. Ming, S. P. Otto, C. L. Peichel, M. W. Pennell, N. Perrin, L. Ross, N. Valenzuela, and J. C. Vamosi. 2014. Tree of Sex: A database of sexual systems. Sci. Data 1:1–8.

Uller, T., I. Pen, E. Wapstra, L. W. Beukeboom, and J. Komdeur. 2007. The evolution of sex ratios and sex-determining systems. Trends Ecol. Evol. 22:292–7.

Uno, Y., C. Nishida, Y. Oshima, S. Yokoyama, I. Miura, Y. Matsuda, and M. Nakamura. 2008. Comparative chromosome mapping of sex-linked genes and identification of sex chromosomal rearrangements in the Japanese wrinkled frog (*Rana rugosa*, Ranidae) with ZW and XY sex chromosome systems. Chromosom. Res. 16:637–647.

Vallender, E. J., and B. T. Lahn. 2004. How mammalian sex chromosomes acquired their peculiar gene content. BioEssays 26:159–69.

Van Doorn, G. S., and M. Kirkpatrick. 2010. Transitions Between Male and Female Heterogamety Caused by Sex-Antagonistic Selection. Genetics 186:629–645.

Van Doorn, G. S., and M. Kirkpatrick. 2007. Turnover of sex chromosomes induced by sexual conflict. Nature 449:909–912.

Van Rheede, T., T. Bastiaans, D. N. Boone, S. B. Hedges, W. W. de Jong, and O. Madsen. 2006. The platypus is in its place: nuclear genes and indels confirm the sister group relation of monotremes and Therians. Mol. Biol. Evol. 23:587–97.

Vernet, N., M. Szot, S. K. Mahadevaiah, P. J. I. Ellis, F. Decarpentrie, O. a Ojarikre, Á. Rattigan, T. Taketo, and P. S. Burgoyne. 2014. The expression of Y-linked Zfy2 in XY mouse oocytes leads to frequent meiosis 2 defects, a high incidence of subsequent early cleavage stage arrest and infertility. Development 141:855–66.

Veyrunes, F., J. Catalan, B. Sicard, T. J. Robinson, J.-M. Duplantier, L. Granjon, G. Dobigny, and J. Britton-Davidian. 2004. Autosome and sex chromosome diversity among the African pygmy mice, subgenus Nannomys (Murinae; Mus). Chromosom. Res. 12:369–82.

Veyrunes, F., P. Chevret, J. Catalan, R. Castiglia, J. Watson, G. Dobigny, T. J. Robinson, and J. Britton-Davidian. 2010. A novel sex determination system in a close relative of the house mouse. Proc. R. Soc. B 277:1049–56.

Veyrunes, F., J. Perez, B. Borremans, S. Gryseels, L. R. Richards, A. Duran, P. Chevret, T. J. Robinson, and J. Britton-Davidian. 2014. A new cytotype of the African pygmy mouse Mus minutoides in Eastern Africa. Implications for the evolution of sex-autosome translocations. Chromosom. Res. 22:533–43.

Veyrunes, F., J. Perez, S. N. C. Paintsil, E. Fichet-Calvet, and J. Britton-Davidian. 2013. Insights into the evolutionary history of the X-Linked sex reversal mutation in *Mus minutoides*: clues from sequence analyses of the Y-linked Sry gene. Sex. Dev. 7:244–52.

Veyrunes, F., P. D. Waters, P. Miethke, W. Rens, D. McMillan, A. E. Alsop, F. Grützner, J. E. Deakin, C. M. Whittington, K. Schatzkamer, C. L. Kremitzki, T. Graves, M. a Ferguson-Smith, W. Warren, and J. a Marshall Graves. 2008. Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. Genome Res. 18:965–73.

Vicoso, B., and D. Bachtrog. 2015. Numerous transitions of sex chromosomes in Diptera. PLoS Biol. 13:e1002078.

Vicoso, B., and D. Bachtrog. 2013. Reversal of an ancient sex chromosome to an autosome in Drosophila. Nature 499:332–5.

Vicoso, B., J. J. Emerson, Y. Zektser, S. Mahajan, and D. Bachtrog. 2013a. Comparative sex chromosome genomics in snakes: differentiation, evolutionary strata, and lack of global dosage compensation. PLoS Biol. 11:e1001643.

Vicoso, B., V. B. Kaiser, and D. Bachtrog. 2013b. Sex-biased gene expression at homomorphic sex chromosomes in emus and its implication for sex chromosome evolution. Proc. Natl. Acad. Sci. U. S. A. 110.

Vuilleumier, S., R. Lande, J. J. M. Van Alphen, and O. Seehausen. 2007. Invasion and fixation of sex-reversal genes. J. Evol. Biol. 20:913–920.

Wainwright, E. N., and D. Wilhelm. 2010. The game plan: cellular and molecular mechanisms of mammalian testis development. Elsevier Inc.

Wang, P. J., J. R. McCarrey, F. Yang, and D. C. Page. 2001. An abundance of X-linked genes expressed in spermatogonia. Nat. Genet. 27:422–6.

Werren, J. H., and L. W. Beukeboom. 1998. Sex Determination, Sex Ratios, and Genetic Conflict. Annu. Rev. Ecol. Sys. 29:233–261.

Wilson, D., and R. Colwell. 1981. Evolution of sex ratio in structured demes. Evolution (N. Y). 35:882–897.

Winking, H., a. Gropp, and K. Fredga. 1981. Sex determination and phenotype in wood lemmings with XXY and related karyotypic anomalies. Hum. Genet. 58:98–104.

Wright, a E., and J. E. Mank. 2013. The scope and strength of sex-specific selection in genome evolution. J. Evol. Biol. 26:1841–53.

Wu, J. B., K. Chen, Y. Li, Y.-F. C. Lau, and J. C. Shih. 2009. Regulation of monoamine oxidase A by the SRY gene on the Y chromosome. FASEB J. 23:4029–38.

Xu, J., and M. Andreassi. 2011. Revresible histone methylation regulates brain gene expression and behaviour. Hor 59:383–392.

Zechner, U., M. Wilda, H. Kehrer-Sawatzki, W. Vogel, R. Fundele, and H. Hameister. 2001. A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? Trends Genet. 17:697–701.

Zhou, Q., and D. Bachtrog. 2012. Sex-specific adaptation drives early sex chromosome evolution in *Drosophila*. Science (80-.). 337:341–345.

Zhou, Q., J. Wang, L. Huang, W. Nie, J. Wang, Y. Liu, X. Zhao, F. Yang, and W. Wang. 2008. Neo-sex chromosomes in the black muntjac recapitulate incipient evolution of mammalian sex chromosomes. Genome Biol. 9:R98.

EVOLUTION D'UN SYSTEME DE DETERMINISME DU SEXE ATYPIQUE CHEZ UN MAMMIFERE, CAUSES ET CONSÉQUENCES.

Le système de déterminisme du sexe des mammifères thériens (XX/XY) est ancien et conservé : toute déviation mène généralement à la stérilité. Cependant, quelques espèces dérogent à la règle. C'est le cas de la souris naine africaine *Mus minutoides*, qui possède un système de déterminisme polygénique où les mâles sont XY, et les femelles XX, XX* ou X*Y (l'astérisque désigne une mutation sur le X, féminisant les embryons X*Y, et apparue il y a presque 1 million d'années). L'évolution d'un tel système est un paradoxe : les femelles X*Y sont censées faire face à des coûts reproductifs importants (perte d'embryons YY, problèmes de méiose…), qui devraient empêcher le maintien de la mutation. Afin de mieux comprendre l'évolution de ce système, nous avons dans un premier temps cherché à identifier les mécanismes évolutifs impliqués dans l'émergence et le maintien du X*. La combinaison d'une approche empirique et d'une étude théorique basée sur des modèles de génétique des populations a permis de mettre en évidence que deux facteurs participent au maintien du X*: un meilleur succès reproducteur des femelles X*Y et la présence de distorteurs de transmission des chromosomes sexuels mâles (leur Y est transmis majoritairement dans les croisements avec des femelles XX et XX* et leur X avec des femelles X*Y). Ce second facteur est certainement à l'origine de l'émergence de ce système. Nous avons ensuite analysé les conséquences de l'évolution de ce système atypique avec trois chromosomes sexuels d'abord sur le phénotype : alors que les trois types de femelles sont indistinguables morphologiquement, les femelles X*Y présentent un comportement masculinisé (elles sont plus agressives et moins anxieuses), puis sur l'évolution de la séquence et de la structure du X et du X* (basé sur des données de séquençage NGS), mettant en évidence que ces chromosomes ont commencé à diverger. Dans l'ensemble, cette étude permet de mieux comprendre les contraintes agissant sur les systèmes de déterminisme du sexe anciens, et les conditions exceptionnelles pouvant réduire ces contraintes permettant ainsi l'évolution d'un nouveau système de déterminisme du sexe. Elle améliore aussi la compréhension de l'impact du complément en chromosomes sexuels sur le phénotype et renseigne sur les forces évolutives agissant sur les chromosomes sexuels dans ce type de système de déterminisme polygénique.

Mots-clés : souris naine africaine, femelles XY, chromosomes sexuels, sex-ratio, modélisation, comportement, génomique

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EVOLUTION OF AN UNUSUAL SEX DETERMINATION SYSTEM IN A MAMMAL, CAUSES AND CONSEQUENCES.

Therian mammals have an extremely conserved XX/XY sex determination system. Their highly differentiated and specialised sex chromosomes are thought to prevent any modification; however, a dozen species harbour unconventional systems. In the African pygmy mouse *Mus minutoides*, all males are XY, and there are three types of females: the usual XX but also XX* and X*Y ones (the asterisk designates a sex reversal mutation on the X chromosome, which evolved almost 1 million years ago). The evolution of such a system is a paradox, as X*Y females are expected to face high reproductive costs (loss of YY embryos, meiotic problems…), which should prevent the maintenance of the mutation. To better understand the evolution of this curious system, we first tried to identify the evolutionary mechanisms involved in the emergence and maintenance of the X*. The combination of empirical data and a theoretical approach based on population genetics models showed that two mechanisms participate in the maintenance of the system: the greater breeding success of X*Y females and the presence of sex chromosome transmission distorters (males transmit their Y more often in crosses with XX or XX* females and their X in crosses with X*Y females), the second mechanism likely being the trigger for the initial spread of the feminising chromosome. We then investigated the consequences of the evolution of this unusual system with three sex chromosomes. First on the phenotype, revealing that despite X*Y females have typical female anatomy and morphology, they resemble males on certain aspects of behaviour: they are more aggressive and less anxious than XX and XX* females. Then on the sequence and structural evolution of the X and X* (based on NGS data), showing that the two chromosomes have started diverging. Altogether, these results shed light on the constraints acting on sex determination systems with highly heteromorphic sex chromosomes and show that rare conditions can loosen these constraints. They also provide valuable insight into the impact of sex chromosome complement on phenotype, and inform on the evolutionary forces acting on sex chromosomes in that kind of polygenic sex determination system.

Key-words: African pygmy mouse, XY females, sex chromosomes, sex-ratio, mathematical modelling, behaviour, genomics