Heredity (2009) 102, 4–15

www.nature.com/hdy

SHORT REVIEW

Speciation through evolution of sex-linked genes

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Identification of genes involved in reproductive isolation opens novel ways to investigate links between stages of the speciation process. Are the genes coding for ecological adaptations and sexual isolation the same that eventually lead to hybrid sterility and inviability? We review the role of sex-linked genes at different stages of speciation based on four main differences between sex chromosomes and autosomes; (1) relative speed of evolution, (2) non-random accumulation of genes, (3) exposure of incompatible recessive genes in hybrids and (4) recombination rate. At early stages of population divergence ecological differences appear mainly determined by autosomal genes, but fast-evolving sex-linked genes are likely to play an important role for the evolution of sexual isolation by coding for traits with sex-specific fitness effects (for example, primary and secondary sexual traits). Empirical evidence supports this expectation but mainly in female-heterogametic taxa. By contrast, there is clear evidence for both strong X- and Z-linkage of hybrid sterility and inviability at later stages of speciation. Hence genes coding for sexual isolation traits are more likely to eventually cause hybrid sterility when they are sex-linked. We conclude that the link between sexual isolation and evolution of hybrid sterility is more intuitive in male-heterogametic taxa because recessive sexually antagonistic genes are expected to quickly accumulate on the X-chromosome. However, the broader range of sexual traits that are expected to accumulate on the Z-chromosome may facilitate adaptive speciation in female-heterogametic species by allowing male signals and female preferences to remain in linkage disequilibrium despite periods of gene flow.
Heredity (2009) **102,** 4–15; doi:10.1038/hdy.2008.93; published online 10 September 2008

Keywords: speciation; sex-linkage; sexual selection; adaptation; genetic incompatibility; genetic mapping

Introduction

Understanding the speciation process remains a challenge in evolutionary biology (Coyne and Orr, 2004; Dieckmann *et al*., 2004; Price, 2007) but in recent decades much progress has been made regarding our knowledge of how reproductive isolation evolves. This focus on the evolution of reproductive isolation probably has its basis in the biological species concept, which defines species as groups of organisms that are reproductively isolated from other such groups (Mayr, 1995). A species is then a group of organisms that share the same gene pool. The rapid development of molecular tools has only recently made it possible to dive into those gene pools and approach the process of speciation at the level of genes. Studies identifying genes underlying reproductive isolation are starting to accumulate, opening up new possibilities to answer central theoretical questions on speciation. At the same time, this development causes novel questions to arise.

Reproductive isolation in sexually reproducing organisms is caused by one or a combination of prezygotic and postzygotic isolation. Prezygotic isolation can be caused by reduced probability of meeting (spatial or temporal isolation), mating (sexual isolation) or successful fertilization (for example due to homogametic sperm preferences or sperm-egg incompatibilities). Postzygotic isolation can be caused by extrinsic factors such as inferior niche adaptation or reduced attractiveness of hybrids or by intrinsic incompatibilities leading to reduced fertility or viability of hybrids. In general, closely related species are reproductively isolated because of many different characteristics that have evolved at different stages of their divergence. The build-up of different forms of isolation often follow a temporal pattern in which ecological differentiation and sexual isolation evolve rapidly and may often precede any form of intrinsic genetic incompatibilities. Reduced fertility of hybrids then invariably precedes the rise of reduced viability.

Why do ecological and behavioural isolation tend to evolve quicker than intrinsic incompatibilities? Are the genes coding for traits underlying ecological adaptations and sexual isolation the same genes that eventually become differentiated enough to cause hybrid sterility and inviability? Genomic studies have a clear role to play in answering such central questions and also in revealing the underlying mechanisms behind general patterns of evolution of genetic incompatibilities such as Haldane's rule. The nature of the genes underlying each form of isolation may be intrinsically linked to their genomic distribution, and both are likely to affect the magnitude and chronology of their role in the speciation process. Just as forms of isolation follow a temporal pattern, the genetics of reproductive isolation may change through time such that genes and genomic regions causing isolation early in the speciation process may differ from those involved later on.

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Received 8 February 2008; revised 16 July 2008; accepted 26 July 2008; published online 10 September 2008

In this review we will ask whether genes causing reproductive isolation are often likely to be disproportionately sex chromosome-linked and we will survey empirical studies to examine the level of support for the hypotheses of the association between sex linkage and speciation. The term 'sex-linkage' typically refers to loci present on the sex chromosomes, which are defined as the chromosome pair that carries the constitutive genes controlling whether an individual develops into a male or a female (Box 1). Hence only organisms with separate sexes, as defined by the relative size of their gametes, have sex chromosomes. However, there are alternative mechanisms such as environmental sex determination (for example, Sarre *et al*., 2004) or haplodiploidy, in which one sex is haploid and the other diploid (for example, Hedrick and Parker, 1997). There are also many species with genetic sex determination but with unidentified sex chromosomes (Ezaz *et al*., 2006; Mank *et al*., 2006a). The most widely known sex chromosome systems are the XX/XY (females are homogametic and have two copies of the X chromosome whereas males are heterogametic and have one X and one Y) and ZZ/ZW (males have two Z chromosomes and females have one Z and one W) systems. In general, the Y or W is smaller (Bull, 1983) and recombination between X/Z and Y/W chromosomes is often restricted to small regions. The fact that the X or Z is hemizygous in the heterogametic sex causes population genetic differences between sex chromosomes and autosomes, and non-random accumulation of particular classes of genes on sex chromosomes. These differences and their link to speciation will be discussed in this article.

Chromosomal sex determination appears to have evolved many times independently in both plants and animals, with male heterogamety being more common. Insects, fishes, amphibians and reptiles contain a mix of male and female-heterogametic taxa, but birds and mammals are unusual because all birds are female-heterogametic and all mammals male-heterogametic (Bull, 1983). This scattered taxonomic distribution and the repeated evolution of sex chromosome systems (for example, Charlesworth, 2002; Mank *et al*., 2006a) and of male and female heterogamety should provide sufficient statistical power for comparative analyses to examine their roles in speciation (for example, Mank *et al*., 2006b).

Sex chromosomes and speciation

Why expect sex-linked genes to have a disproportionately large influence on reproductive isolation?

In the absence of differences in relevant features between sex chromosomes and autosomes, their influence on speciation would be expected to be proportional to their contribution to the genome in terms of size or the number of genes they carry (Ritchie and Phillips, 1998). However, there are at least four main factors that may lead to differences between sex chromosomes and autosomes in their relationship with speciation, all of which are linked to hemizygosity (Figure 1): (1) relative speed of evolution, (2) non-random accumulation of genes, (3) exposure of incompatible recessive genes in hybrids and (4) recombination rate. Below we will discuss, in the light of different mechanisms of speciation, whether components of reproductive isolation are expected to be determined by sex-linked genes to a larger extent than is expected by chance. Our main aim is to pinpoint how the four outlined differences between sex chromosomes and autosomes may influence the relative role of sex-linked genes at different stages of the speciation process. We focus on sex determination systems with stable heteromorphic sex chromosomes and most of the following discussion will be of the X/Z chromosome because it often contains more genes than

Box 1 Sex chromosomes and sex determination

The evolution of identifiable heteromorphic sex chromosomes is initiated by the spread of a sex-determining gene (SDG). This occurs when a new mutation at a locus leads all its carriers to become the same (subsequently heterogametic) sex, with the chromosome carrying this mutation becoming the Y/W chromosome (see main text). In eutherian mammals, for example, the development of males is controlled by the SRY gene found only on the Y chromosome. New sex chromosome systems can evolve from a co-sexual ancestor (with both sexes in the same individual), from environmental or multifactorial sex-determination, or from an existing sex chromosome system. In the latter case, the ancestral X/Z becomes an autosome and the ancestral Y/W may disappear (van Doorn and Kirkpatrick, 2007). Sex-chromosomal heteromorphism then increases over time. Sexually antagonistic loci with alleles favoured in the heterogametic sex but disfavoured in the homogametic sex should accumulate around the SDG on the Y/W chromosome, because reduced recombination with this locus will lead them to be present less often in the homogametic sex (Rice, 1996). This in turn will favour reduced recombination over a greater region on the Y/W chromosome. Reduced recombination then causes heteromorphism through erosion of the Y/W chromosome, because of either Muller's ratchet (gradual accumulation of deleterious mutations; Charlesworth (1978)) or genetic hitchhiking of deleterious mutations with beneficial mutations (Rice, 1987). Interestingly, not only should sexually antagonistic loci accumulate around existing SDGs, but new SDGs are more likely to become established when the mutation arises in a gene linked to existing sexually antagonistic loci (van Doorn and Kirkpatrick, 2007).

Despite the location of the SDG being part of the definition of sex chromosomes (Charlesworth *et al*., 2005), in many taxa, including birds (Ellegren, 2001), it is not clear whether the Y/W chromosome is involved in sex determination, whereas in *Caenorhabditis elegans*, *Drosophila melanogaster* and the flowering plant *Rumex acetosa*, the lack of involvement of the Y/W chromosome has been confirmed (Cline, 1993; Löve, 1969; Hsu and Meyer, 1993). In other taxa such as many Orthopteran insects there is no Y/W chromosome (Bull, 1983) with the heterogametic sex carrying only a single X (termed XO). Sex determination may in these cases be initiated by an interaction between X/Z chromosome dosage and an autosomal SDG.

Variation among taxa in the degree of heteromorphy of the sex chromosomes affects hemizygosity and may therefore influence the relationship between sex linkage and speciation. For example in some taxa the heterogametic sex is not hemizygous (they are XXY or ZZW). Furthermore, in recently evolved sex chromosomes large areas of homology and recombination may remain between the X/Z and Y/W chromosomes (Charlesworth *et al*., 2005), so that only restricted regions are absent from the homogametic sex. The degree of hemizygosity may therefore be linked to the rate of sex chromosome turnover (van Doorn and Kirkpatrick, 2007) because higher turnover should be linked to relatively younger sex chromosome systems. This may in turn affect the role of sex chromosomes in the evolution of reproductive isolation.

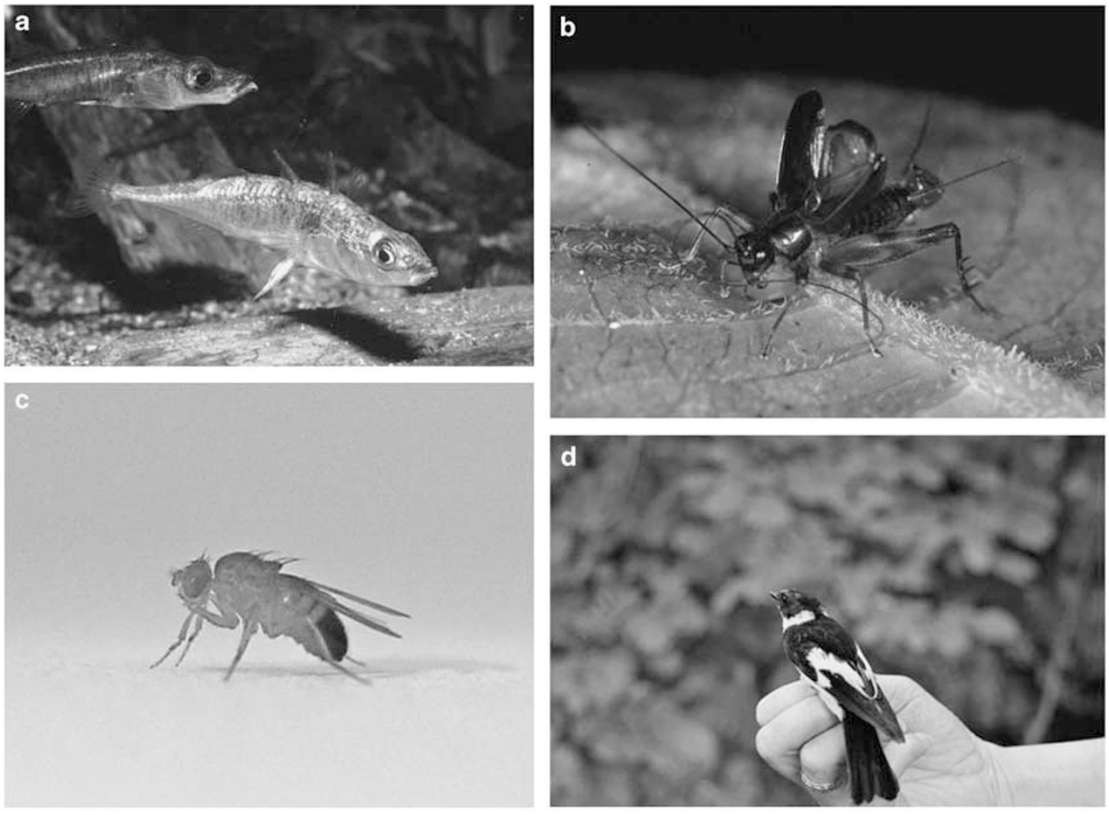

Figure 1 Reasons to consider the role of sex linkage in speciation: (**a**) Threespine sticklebacks, *Gasterosteus aculeatus*. Non sex-biased ecological selection on pre-existing genetic variation has led, in this case, to the spread of an autosomal variant reducing armour plating in freshwater limnetic fishes. The female in the photo (below) is fully plated, with the plates visible down the lateral line. The male (above) is the Enos Lake limnetic species, which has reduced armor. Ectodysplasin is undoubtedly the main genetic factor underlying the difference in plates between the two forms (photo credit Ernie Cooper, World Wildlife Fund Canada, ecooper@wwfcanada.org); (**b**) A male of the Hawaiian cricket *Laupala paranigra*. Differences in male calling song between this species and *L. kohalensis* are controlled by many genes of small to moderate effect, which are not disproportionately found on the X chromosome (photo credit Kerry Shaw); (**c**) A male of the fruit fly *Drosophila melanogaster*. Although sexual isolation between populations of this species is mainly controlled by autosomal factors, interspecific studies of *D. melanogaster* and the closely related *D. simulans*, *D. mauritiana* and *D. sechellia* reveal that the X diverges faster than autosomes, an excess of male sterility factors are recessive and they are also disproportionately located on the X chromosome (photo credit Sandra Hangartner). (**d**) A 'typical' hybrid between pied and collared flycatchers (*Ficedula hypoleuca* and *F. albicollis* respectively). Its plumage pattern is intermediate with a clearly broken collar and grey-brown elements in the black back. A male species recognition plumage trait, female preference and hybrid sterility factors are all located on the Z chromosome. Such non-random accumulation of traits involved in reproductive isolation onto a single chromosome should facilitate sympatric speciation and reinforcement by reducing recombination between them.

the Y/W (Box 1), increasing its possible influence on speciation.

Early ecological divergence

The relative speed of evolution should differ for ecological traits on sex chromosomes versus autosomes: Rapid and strong ecological divergence often occurs during the earliest stages of speciation either because subdivided populations adapt to different environments in allopatry or because there is strong competition over one or more resource types causing disruptive natural selection in sympatry (Dieckmann and Doebeli, 1999; Schluter, 2000; Gavrilets, 2004). Selection against intermediate phenotypes is therefore initially largely dependent on their failure to effectively utilize either parental niche. Are there any reasons to expect that the genes coding for traits that cause adaptation to different feeding niches and hence disruptive selection, such as beak size in Darwin's Finches, should often be disproportionately sex-linked?

There are two reasons why the earliest stages of ecological divergence may involve relatively few sex-linked genes: the strong influence of existing genetic variation relative to new mutations and equivalent selection in the two sexes. Although new beneficial mutations that are at least partially recessive are expected to accumulate on sex chromosomes because they are exposed to selection in the heterogametic sex (Charlesworth *et al*., 1987), novel selection on standing genetic variation previously maintained at mutation–selection balance will lead to greater accumulation of changes on autosomes (Orr and Betancourt, 2001). This is because the X/Z chromosome typically has a smaller effective population size than autosomes (Whitlock and Wade, 1995), which means that previously deleterious mutations will initially be present at lower frequency. At the same time, genes that do not have sex-biased expression or sexually antagonistic selection, such as those underlying many ecological traits with equivalent effect on fitness in the two sexes, are not expected to

Table 1 Recent studies of sex linkage of isolating mechanisms[a]

Isolating mechanism	*Species*	*Trait*	*Heterogametic sex*	*Loci mapped?*[b]	*Gene(s) identified?*	*X/Z effect*[c]	*References*
Ecological	*Geospiza fortis, G. magnirostris, G. fuliginosa, G. scandens, G. conirostris, G. difficilis, G. olivacea*	Beak morphology	Female	No	Yes	No[d]	Abzhanov *et al*. (2004, 2006)
	Chaetodipus intermedius	Coat colour	Male	No	Yes	No[d]	Nachman *et al*. (2003)
	Gasterosteus aculeatus	Armour plating	Male	Yes	Yes	No	Peichel *et al*. (2004); Colosimo *et al*. (2005)
	Gasterosteus aculeatus	Pelvic skeleton	Male	Yes	Yes	No	Shapiro *et al*. (2004)
Prezygotic isolation	*Drosophila lummei* × *D. virilis*	Male pheromone	Male	Yes	No	Yes^{+}	Liimatainen and Jallon (2007)
	Drosophila mauritiana × *D. simulans*	Sexual isolation (various traits)	Male	Yes	No	Yes^{-}	Moehring *et al*. (2004)
	D. melanogaster African × Cosmopolitan	Female pheromone	Male	Yes	Yes	No	Takahashi *et al*. (2001)
	D. melanogaster Zimbabwe × Cosmopolitan	Sexual isolation (male and female effects)	Male	Yes	No	Yes^{-}	Wu *et al*. (1995); Hollocher *et al*. (1997b); Ting *et al*. (2001)
	D. melanogaster African × Cosmopolitan	Male pheromone	Male	Yes	No	Yes	Scott and Richmond (1988)
	D. melanogaster African × Cosmopolitan	Male courtship song	Male	Yes	No	No	Colegrave *et al*. (2000)
	D. melanogaster Zimbabwe × Cosmopolitan	Mated female gene expression differences	Male	Yes	Yes	Yes$^{=}$	Michalak *et al*. (2007)
	D. pseudoobscura × *D. persimilis*	Sexual isolation (various traits)	Male	Yes	No	Yes^{-}	Noor (1997)
	D. santomea × *D. yakuba*	Sexual isolation (various traits)	Male	Yes	No	Yes^{-}	Moehring *et al*. (2006)
	D. sechellia × *D. simulans*	Male courtship song	Male	Yes	No	No	Gleason and Ritchie (2004)
	D. sechellia × *D. simulans*	Female pheromone	Male	Yes	No	Yes^{-}	Gleason *et al*. (2005)
	Ephippiger ephippiger song races	Male calling song	Male	No	No	Yes^{+}	Ritchie (2000)
	Ephippiger ephippiger song races	Female preference	Male	No	No	No	Ritchie (2000)
	Ficedula albicollis × *F. hypoleuca*	Female preference	Female	No[e]	No	Yes	Sæther *et al*. (2007)
	Ficedula albicollis × *F. hypoleuca*	Male plumage	Female	No[e]	No	Yes	Sætre *et al*. (2003)
	Heliconius cydno × *H. melpomone*	Wing colour pattern	Female	Yes	No	No	Naisbit *et al*. (2003)
	Laupala kohalensis × *L. paranigra*	Male song pulse rate	Male	Yes	No	Yes$^{=}$	Shaw *et al*. (2007)
Hybrid sterility	*Drosophila mauritiana* × *D. sechellia*	Male sterility	Male	Yes	No	Yes^{+}	Masly and Presgraves (2007)
	D. mauritiana × *D. simulans*	Male sterility	Male	Yes	No	Yes^{+}	True *et al*. (1996); Tao *et al*. (2003)
	D. mauritiana × *D. simulans*	Male sterility	Male	Yes	Yes	Yes	Ting *et al*. (1998)
	Ficedula albicollis × *F. hypoleuca*	Male and Female sterility	Female	No[e]	No	Yes	Svedin *et al*. (2008)
	Heliconius cydno × *H. melpomone*	Female sterility	Female	No	No	Yes	Naisbit *et al*. (2002)
	H. melpomone geographic races	Female sterility	Female	No	No	Yes	Jiggins *et al*. (2001)
	Mus mus domesticus × *M. m. molossinus*	Male sterility	Male	Yes	No	Yes	Oka *et al*. (2004, 2007)
Hybrid inviability	*Drosophila melanogaster* × *D. simulans*	Viability	Male	Yes	No	Yes	Presgraves (2003)
	D. melanogaster × *D. simulans*	Male viability	Male	Yes	Yes	Yes	Summarized in Presgraves (2006)
	D. melanogaster × *D. simulans*	Male viability	Male	Yes	Yes	Yes	Presgraves *et al*. (2003)
	Xiphophorus helleri × *X. maculates*	Viability	Male	Yes	Yes	Yes	Schartl (1988); Zechel *et al*. (1988); Wittbrodt *et al*. (1989)
Gene misexpression	*Drosophila mauritiana* and *D. sechellia* × *D. simulans*	Under/overexpression in hybrids	Male	Yes	Yes	Yes^{-}	Moehring *et al*. (2007)

[a]Studies included in Ritchie and Phillips (1998) and Prowell (1998) are not included here. Only postzygotic isolation studies from the past 10 years are included, unless the gene was identified or the proportion of sex linkage tested. For brevity ecological isolation includes only those studies in which both a putative role in natural selection for the trait and a relevant gene have been identified.
[b]Mapped to a finer resolution than the presence/absence of sex linkage.
[c]If disproportionate sex linkage is tested the result is indicated by $^{-}$(large autosomal effect), $^{=}$(no apparent pattern), $^{+}$(large X/Z effect).
[d]Presence or absence of sex linkage inferred from the gene location in a closely related species (see main text).
[e]Although other studies involved laboratory crosses and linkage mapping or introgression studies, the flycatcher study involved analysis of pedigrees and cross-fostering experiments in a natural hybrid zone.

accumulate more often than by chance on sex chromosomes. However, once new mutations begin to accumulate, the influence of sex-linked genes with additive effects on ecological traits may increase because of greater exposure to selection (Charlesworth *et al*., 1987) and increase or decrease owing to sex- (or sex chromosome-) biased mutation rates. In birds, male-biased mutation rates (Hurst and Ellegren, 1998) would mean faster evolution of Z-linked genes (Kirkpatrick and Hall, 2004) but male-biased mutation in mammals means relatively slower evolution of X-linked genes (Kirkpatrick and Hall, 2004; Vicoso and Charlesworth, 2006).

A useful first step towards an understanding of the molecular genetic basis of the ecological adaptations that underpin extrinsic postzygotic isolation is to pinpoint the molecules involved in embryonic developmental pathways that underlie the specific morphological structures associated with niche use. This is because relatively few changes in such molecules may lead to relatively large changes in the phenotype. Table 1 lists studies in which genes involved in niche use differentiation have been identified, along with studies revealing the effects of sex-linked genes on prezygotic isolation, hybrid sterility and hybrid inviability. An elegant combination of a candidate gene approach and microarray analysis has recently revealed molecules associated with different outcomes of embryonic development of the beak in Darwin's finches. A single factor, the bone morphogenetic protein 4 precursor (BMP4), appears to promote a deeper and wider beak (Abzhanov *et al*., 2004) whereas higher local expression of Ca^{2+} calmodulin-dependent protein kinase phosphatase (CaM) is associated with elongation of the beak (Abzhanov *et al*., 2006). Variation in beak shape along these different axes neatly matches different species of Darwin's Finches' ability to feed on seeds of different sizes (Bowman, 1961). Furthermore, changing selection pressures have been found to lead to adaptive changes in beak shape of the medium ground finch (*Geospiza fortis*), over a relatively short time period (Grant and Grant, 2002). The locations of the genes are unknown in Darwin's Finches, but in the chicken, the corresponding genes are found on chromosome 15 (CaM) and on chromosome 5 (BMP4) (Hillier *et al*., 2004). Given the stable karyotype of birds these genes most likely remain autosomal in the finches. Similarly, Nachman *et al*. (2003) used a candidate gene approach to reveal the genetic basis of adaptive changes in coat colour of rock pocket mice. The melanocrine-1-receptor gene (Mc1r, located on chromosome 8 in *Mus*; MGSC, 2002) was, in one study population, found to be associated with variation in cryptic coloration (Nachman *et al*., 2003). These mice are at a particularly early stage of (potential) divergence with neutral gene flow between colour morphs apparently unrestricted, but with good evidence of selection maintaining sequence differences at the Mc1r locus between populations on light and dark-coloured rocks (Hoekstra *et al*., 2004). Another appealing example of a molecular approach for examining the genetic basis of an adaptive morphological shift comes from studies on sticklebacks. The ectodysplasin (Eda) gene has been found to be associated with the typical armour plate reduction that occurs when marine threespine sticklebacks colonize freshwater lakes and streams (Colosimo *et al*., 2005). This gene is autosomal in threespine sticklebacks being located on linkage group 4 (Colosimo *et al*., 2005), whereas the putative nascent sex chromosomes correspond to linkage group 19 (Peichel *et al*., 2004). Because this allele is present at low frequency in ancestral marine populations (Colosimo *et al*., 2005) adaptation to the freshwater environment does not depend on novel mutations. To summarize, based on theoretical expectations and on a few pioneering empirical studies we conclude that there is no current support for an over-representation of sex-linked genes in the earliest stages of ecological divergence.

Evolution of sexual isolation

Genes involved in sexual isolation are expected to accumulate non-randomly on sex chromosomes: Ecological divergence often needs to be coupled with evolution of prezygotic (or sexual) isolation to avoid the homogenizing effect of gene exchange. This is the case when divergence occurs in sympatry or when populations come into secondary contact before they have become completely reproductively isolated. Sexual isolation is likely to be an important barrier because it acts early in the reproductive cycle (Coyne and Orr, 2004) and closely related species indeed often differ markedly in sexually selected traits (West-Eberhard, 1983; Price, 1998; Panhuis *et al*., 2001). Prezygotic isolation does not always rely on species-specific display traits and preferences for those traits. Apart from isolation through species-specific ecological habits (for example, Via, 2001), interbreeding may also be prevented through divergence of primary sexual organs. Primary sex organs may evolve in response to both natural and sexual selection, including components of sexually antagonistic selection causing rapid evolution and divergence (Gavrilets, 2000; Arnqvist and Rowe, 2004; Hayashi *et al*., 2007). Finally, postmating prezygotic isolation may arise through divergence of reproductive proteins, which are involved in gamete recognition systems preceding the fusion of gametes. The evolution of these proteins may, at least partly, also be driven by an antagonistic arms race between males and females causing very rapid evolution (Rice and Holland, 1997; Panhuis *et al*., 2006). Although phenotypes contributing to ecological isolation tend to be equally expressed and share similar selective pressures across both sexes, such as the examples given (in section, Early ecological divergence) both primary (for example sex organs, sperm-egg interaction proteins) and secondary (for example, display traits) sexual characters have sex-specific fitness effects and often sex-biased expression. Such features increase the likelihood of intraspecific sex-linkage through causing accumulation of these loci on sex chromosomes (Ellegren and Parsch, 2007), but also because existing sexually antagonistic loci tend to attract new sex-determining genes (Box 1; van Doorn and Kirkpatrick, 2007). With intense selection on males, which is expected for traits involved in reproductive competition, female fitness can be adversely affected by correlated evolution when sexually antagonistic selection occurs. Sexual conflict through intra-locus sexually antagonistic selection should favour accumulation of genes on the sex chromosome that is less often found in the sex with lower fitness, or of recessive genes on the X/Z chromosome that are favoured in the heterogametic sex (Rice, 1984). Hence, recessive alleles coding for traits

favoured in heterogametic males (for example, mammals) are expected to accumulate on the X chromosome because they are only rarely exposed to antagonistic selection in females. By contrast, sexually antagonistic alleles that are dominant and favour homogametic males (for example, birds) are expected to accumulate on Z because Z spends two times as much time in males (Rice, 1984). For X/Z-linked genes modifiers of gene expression that cause reduced expression in the sex with lower fitness and/or increased expression in the sex with higher fitness should then accumulate. If these regulatory modifiers are *cis*-acting (found within or close to the coding gene they regulate), they will also tend to accumulate on the X/Z chromosome. Evidence for disproportionate sex linkage of sexually selected traits within species is mixed (for example, Reinhold, 1998; Wolfenbarger and Wilkinson, 2001; Gleason *et al*., 2002; Lindholm and Breden, 2002; Fitzpatrick, 2004a; Huttunen *et al*., 2004). In Poecilid fishes male secondary sexual traits are typically sex-linked, although more often found on the Y only or both X and Y than on the X alone (Lindholm and Breden, 2002). In a broader taxonomic review, Reinhold (1998) found evidence for excessive sex linkage of sexually selected traits. Conversely Fitzpatrick (2004a) revealed that sexually selected genes are distributed across the genome in proportion to the genomic contribution (number of genes present) of each chromosome in *Drosophila melanogaster*, and Wolfenbarger and Wilkinson (2001) found the same for eyespan in male stalk-eyed flies.

However, whether genes coding for mate preferences should be excessively sex-linked is a more open question. Relatively little is known about the genetic basis of species-specific mate preferences (Table 1; Ritchie and Phillips, 1998). Female preference is Z-linked in the moth *Utetheisa ornatrix* (Iyengar *et al*., 2002), but is not X-linked in the bush cricket *Ephippiger ephippiger* (Ritchie, 2000; Table 1). In general, polygenic behaviours are less likely to be excessively sex-linked because their evolution is expected to have been gradual rather than rapid (Ritchie and Phillips, 1998), reducing the impact of the relatively faster evolution of genes on sex chromosomes. Fisherian runaway sexual selection promotes the accumulation of novel male ornament genes on Z chromosomes but not on X chromosomes (Reeve and Pfennig, 2003) and runaway sexual selection is itself promoted when female preferences are Z-linked (Kirkpatrick and Hall, 2004). Selection for female counter-adaptations would also be more efficient than on autosomes when beneficial alleles are recessive (Charlesworth *et al*., 1987) further strengthening Z- but not X-linkage (because female preferences or counter-adaptations are not favoured or not expressed in males). On the other hand, X-linkage of female preferences and autosomal inheritance of male ornaments favours sexual selection through 'good genes' (Kirkpatrick and Hall, 2004), which may equally lead to exaggerated ornaments and rapid divergence. An alternative evolutionary pathway is that display traits initially diverge between populations through processes other than mate choice (for example, through male–male competition) and then become linked with corresponding mate preferences at a stage when there are high genetic costs of hybridization or direct benefits associated with species recognition, for example during periods of secondary contact after divergence in allopatry. With such a lack of genetic correlations between male and female parts of the sexual signalling system during divergence (for example, Qvarnström *et al*., 2006), there is again little expectation of strong sex-linkage of mate preference. Thus, overall theory predicts that genes coding for traits with sex-specific fitness effects should be tightly linked with the evolution of the sex chromosomes and *vice versa*, but some important predictions differ between male- and female-heterogametic taxa.

Previous review articles have concluded an over-representation of both X-linkage (Reinhold, 1998; Lindholm and Breden, 2002) and Z-linkage (in Lepidoptera) of sexually selected traits (Prowell, 1998). We list additional recent empirical tests of sex linkage of sexual isolation traits in Table 1 and conclude that there is little evidence for an over-representation of X-linkage of these traits. One particularly well-studied case of the very earliest stages of divergence reveals few X-linked effects. Begun and Aquadro (1993) discovered *Drosophila melanogaster* populations in Zimbabwe that were genetically differentiated from other populations, particularly those from outside sub-Saharan Africa. Subsequent studies revealed strong sexual isolation between these strains (Wu *et al*., 1995; Hollocher *et al*., 1997a, b) but little postzygotic isolation (Alipaz *et al*., 2005), indicating an early stage of speciation. Genetic analyses revealed that both male and female traits involved in assortative mating were most strongly influenced by chromosome 3 and least of all by the X chromosome (for example, Wu *et al*., 1995; Hollocher *et al*., 1997b; Ting *et al*., 2001; Michalak *et al*., 2007).

Another interesting example of a recent divergence lacking a large influence of X-linked genes on sexual isolation comes from a suite of elegant studies on Hawaiian crickets. The strikingly rapid (and possibly sympatric) radiation among the Hawaiian *Laupala* crickets (Mendelson and Shaw, 2005) is probably driven by divergence in calling song and preference (Shaw and Parsons, 2002). By using a combination of QTL studies and investigations of segregation patterns in F2 hybrids between two closely related species of *Laupala* crickets Shaw *et al*. (2007) found that many genes with small effects were involved in the determination of song and that there was no disproportionate large X effect. To summarize, based on theoretical expectations and on interspecific empirical studies (Table 1) there is more evidence suggesting a large Z effect than a large X effect in the divergence of sexual signalling systems. We suggest that sexually antagonistic loci coding for primary sexual traits should strongly influence sexual isolation because they are directly involved in reproduction, should accumulate on sex chromosomes and should evolve relatively rapidly once there, potentially giving them a large influence on the development of reproductive isolation. The influence of secondary sexual traits may be more variable, but stronger in female heterogametic taxa (Figure 1).

Evolution of genetic incompatibility

Hemizygosity means exposure of incompatible recessive genes in hybrids: Speciation is likely to begin through the development of extrinsic isolating mechanisms, and this isolation will then allow the build-up of genetic

differences leading to intrinsic incompatibilities. Although studies suggest that divergence in sexual and ecological isolation typically involves additive genetic differences (Coyne and Orr, 2004), intrinsic postzygotic isolation normally involves epistatic (non-additive) interactions. Most genetic models of the evolution of intrinsic postzygotic isolation (hybrid infertility or inviability) rely on epistatic interactions between genes, the most classic being the Dobzhansky–Muller model (Dobzhansky, 1940; Muller, 1940). The basic version of this model builds upon an evolutionary change in only two loci. A population splits into two parts and mutations at different loci become fixed in these two new isolated populations, without either causing a reduction in fitness within its own population (hence avoiding the crossing of an 'adaptive valley'; Gavrilets, 2004). Because these new genes are lacking a co-evolutionary history they may function poorly together, resulting in fertility and viability reductions of hybrids between the two populations upon secondary contact. Empirical evidence for the importance of epistatic interactions in speciation comes, for example, from the observation known as Haldane's rule (greater fitness reduction of hybrids of the heterogametic sex) and from patterns of 'hybrid breakdown' (F2 hybrids having lower fitness than F1 hybrids). Haldane's rule is generally followed across all taxa studied and two hypotheses predominate in explaining this pattern (Coyne and Orr, 2004). The dominance hypothesis, which builds on the Dobzhansky–Muller model, suggests that sterility and inviability in the heterogametic sex is caused by recessive alleles that, although they may be randomly distributed throughout the genome, only cause problems (through interactions with other loci) when X/Z-linked and hemizygous (Muller, 1940). Alternatively, the faster male hypothesis suggests that males evolve faster than females, typically due to more intense reproductive competition in males and this naturally leads to a more rapid build-up of isolation in male-limited traits that are highly likely to be involved in reproductive isolation (Wu and Davies, 1993; Wu *et al*., 1996). This latter hypothesis does not predict Haldane's rule in female-heterogametic taxa. There are several additional theories such as 'faster-X,' which assumes fast evolution and hence divergence of X-linked genes (Charlesworth *et al*., 1987). Moreover, genomic studies suggest that there may be a link between the evolution of hybrid sterility and interlocus antagonistic co-evolutionary arms races taking place between genes within the same genome. Selfish meiotic-drive alleles may distort the sex ratio to their own advantage leading to their increase in frequency in the population, which in turn causes selection for genes that suppress drivers and restore the sex ratio (Pomiankowski and Hurst, 1993). Such sex ratio distorters are typically found on the X chromosome (Hurst and Pomiankowski, 1991; Jaenike, 2001). When two diverged populations hybridize, the drivers may escape the control of the suppressors and cause male sterility. Orr *et al*. (2006) found that the same genes influence hybrid sex ratios and hybrid sterility in crosses between two *Drosophila* species, although Masly and Presgraves (2007) found no supporting evidence in another *Drosophila* study.

It is important to recognize that these hypotheses are not mutually exclusive but may very well work in concert. For example, faster evolution of male reproductive traits (supporting the faster-male hypothesis) and their non-random accumulation on the sex chromosomes (see section, Evolution of sexual isolation) may augment fast divergence of sex-linked genes (supporting the faster-X hypothesis; see section, Early ecological divergence and the exposure of incompatible recessive genes in hybrids (the dominance hypothesis; this section). Most importantly, these hypotheses are all consistent with the non-random build-up and/or expression of intrinsic isolation on the X/Z chromosome, and this is well-supported by empirical studies (Table 1). Studies of disproportionate X/Z effects that account for recessivity of hybrid dysfunction remain few and only for male-heterogametic taxa, but appear to support a large X effect for sterility (Table 1). True *et al*. (1996), Tao *et al*. (2003) and Masly and Presgraves (2007) have provided convincing evidence that male sterility factors in crosses between *Drosophila simulans*, *D. mauritiana* and *D. sechellia* occur at a higher density on the X chromosome than on autosomes and at a much higher density than female sterility factors, supporting faster male evolution. Their results also reveal an excess of recessive incompatibilities, supporting the dominance theory, but leave open the question of whether faster-X evolution is involved. Masly and Presgraves (2007) suggest that disruption of dosage compensation (transcription is normally increased on the X in male *Drosophila* to match that in females) or of X-inactivation during spermatogenesis are more likely mechanistic explanations for the large-X effect than faster-X evolution. However, despite previous studies providing no statistical support for faster-X evolution (Begun and Whitley, 2000; Betancourt *et al*., 2002; Thornton *et al*., 2006), Begun *et al*. (2007) recently found strong evidence for faster-X in *D. simulans*, *D. melanogaster* and *D. yakuba*, which they suggest may be because of higher mutation rates on the X chromosome combined with more rapid adaptive divergence. The dominance theory is strongly supported for the more slowly-evolving hybrid inviability (Coyne and Orr, 2004). For studies in which inviability genes or genomic regions have been identified, it is typically caused by epistatic interactions between a small number of X-linked and autosomal loci (Table 1). No similarly detailed studies have been carried out in female-heterogametic taxa (Table 1), although there is evidence of fast-Z evolution in birds (Mank *et al*., 2007) and Lepidoptera (Prowell, 1998). In conclusion, several specific characteristics of sex chromosomes add up to make them hotspots for the accumulation and expression of genes that are incompatible between diverged populations.

The maintenance of co-adapted gene complexes

Disproportionate sex linkage may shelter co-adapted gene complexes from introgression: Speciation is often onset by a geographical split of populations. However, because the rise of intrinsic genetic incompatibility is a very slow evolutionary process, for example, 2–4 million years for evolution of hybrid inviability in mammals, (Fitzpatrick, 2004b) and more than 10 million years for evolution of hybrid inviability in birds (Price and Bouvier, 2002) many diverged populations come into contact when they are, at least partly, compatible. When

sexual isolation is incomplete, selection against hybrids may reinforce assortative mating (Dobzhansky, 1940; Lemmon and Kirkpatrick, 2006), which in turn allows the further build-up of genetic incompatibilities. Furthermore, a balance between gene flow and selection against hybrids often leads to the formation of narrow hybrid zones, reducing recombination and therefore allowing divergence between populations either side of the zone to continue (parapatric speciation; Kondrashov, 2003). Clines of sex-linked loci are often narrower than those of autosomal loci, for example in the house mouse (Tucker *et al*., 1992; Dod *et al*., 1993, 2005; Raufaste *et al*., 2005; Macholán *et al*., 2007), indicating either that selection against hybrids is more efficient on the X/Z chromosome or that it is more likely to contain genes selected against in hybrids. Either way, recombination in parapatry is lower for X/Z-linked loci than autosomal loci, increasing their potential for divergence. The feasibility of reinforcement has been questioned because genetic recombination between the co-adapted traits (that is, female preference, male sexual trait and genes causing low fitness of hybrids) will counteract speciation (Felsenstein, 1981). The absence of X/Z recombination at many loci in the heterogametic sex may often reduce average recombination rate in the X/Z chromosome, so that sets of differentiated loci involved in reproductive isolation have a greater probability of being maintained in linkage disequilibrium if they co-occur on the X/Z chromosome. Theoretical models show that sex linkage of traits involved in both pre- and postzygotic isolation may be a favourable condition for adaptive speciation (such as reinforcement, Servedio and Sætre, 2003; Lemmon and Kirkpatrick, 2006). Sæther *et al*. (2007) tested this central prediction by using a combination of field experiments, molecular markers to assign parental species combinations, and long-term breeding data from hybrid zones of two closely related species of *Ficedula* flycatchers. They found that species recognition was Z-linked, as were male plumage traits and genes causing low fitness in hybrids (Table 1). These findings, together with the fact that sex-linked genes are often associated with adaptation and speciation in Lepidoptera (Prowell, 1998; Ritchie and Phillips, 1998) suggest that female heterogamety may facilitate adaptive speciation.

How general is the conclusion that reduced recombination of sex chromosomes should facilitate non-allopatric speciation? Humans and chickens have somewhat lower average recombination rates on the sex chromosomes than autosomes (Sundström *et al*., 2004; Butlin, 2005). Conversely, compensation in the form of greater chiasma number (and hence more points for crossing over) may occur, as in mice (Burt *et al*., 1991), equalizing recombination rates between sex chromosomes and autosomes. In species with achiasmate meiosis and hence no recombination in the heterogametic sex, such as *Drosophila melanogaster*, the average recombination rate in the absence of compensation is in fact higher on the X/Z chromosome than on autosomes because it is more often found in the homogametic sex (Butlin, 2005). Indeed recombination rates are higher on the X chromosome in *D. melanogaster* (Butlin, 2005). Furthermore, genomic regions with low intraspecific recombination rates should suffer greater accumulation of deleterious mutations because of Muller's ratchet and to hitchhiking with beneficial mutations, as is the case with Y/W chromosomes (Charlesworth, 1978; Rice, 1987). This may mean that genomic regions reducing recombination between diverging populations without having similar effects within population are most likely to be involved in speciation (Butlin, 2005). For example, chromosomal rearrangements fixed in one population may decrease gene flow with a second population lacking the rearrangements for segments of the genome, within which accumulation of incompatibilities and locally adapted genes can consequently gradually occur (Noor *et al*., 2001; Rieseberg, 2001; Navarro and Barton, 2003; Chang and Noor, 2007). Sex chromosomes are generally the most conserved elements of the mammalian (for example, Brudno *et al*., 2004) and bird (for example, Backström *et al*., 2006) karyotypes. However, when chromosomal changes involve the sex chromosomes they often lead to complete sterility of hybrids (King, 1993; but see for example, Veyrunes *et al*., 2007). Thus, there is a general agreement that accumulation of speciation genes in genomic regions with low interspecific recombination rates would facilitate the formation of species when divergent populations are experiencing periods of gene flow. To what extent sex chromosomes (or parts of them) function as such 'speciation islands' boils down to an empirical question.

Conclusions and future directions

A high rate of genetic divergence, the development of linked groups of loci sheltered from recombination, and multiple pleiotropic effects of single loci on reproductive isolation all facilitate speciation. This combination of factors may differ markedly between regions of the genome, and regions of the genome involved in reproductive isolation may differ between taxa and between temporal stages of the speciation process. For this reason, genomic studies can tell us much more about speciation than merely the identity of speciation genes.

What role are sex-linked genes playing at different stages of speciation? We outline one scenario we consider to be particularly plausible. Clearly, an increased rate of evolution increases the speed of divergence between subdivided populations, so parts of the genome that evolve faster should also diverge faster. During the initial stages of speciation, when a population shifts ecological adaptations, changes in the frequency of pre-existing alleles (for example, the stickleback example outlined in section, Early ecological divergence) are likely to have an important function, reducing the influence of sex chromosomes at this earliest stage. Once a population has entered a new environment, for example, colonized an island or started to utilize a new niche in sympatry, reproductive traits will diverge relatively rapidly (for example, due to sexual conflict). Because this divergence in sexual traits depends on novel mutations, which are most likely to become fixed on the sex chromosomes because of their sexually antagonistic fitness effects and greater exposure to selection, the influence of sex chromosomes will steadily increase. Traits involved in reproduction are also likely to contribute directly to reduced hybrid fertility, so such genomic regions will become incompatible between populations (according to the Dobzhansky–Muller model) relatively quickly, and their influence will be greater still because of increased expression in the heterogametic sex. As these

incompatible genomic regions are found on the sex chromosomes—which often recombine less in sympatric hybridizing populations (for example, Sætre *et al*., 2003) and hybrid zones (because of narrower cline widths and hence stronger barriers to gene exchange)—genes coding for population-specific adaptations may then start to accumulate close by these regions during periods of sympatry. At this later stage of the speciation process, selection against hybrids may directly lead to the evolution of fine-tuned species recognition systems in sympatry because the relevant traits are sheltered from recombination. According to this scenario, sex chromosomes should have an important function in the speciation process. Our review illustrates that evidence so far suggests that traits involved in sexual isolation are not typically disproportionately X-linked but they may be disproportionately Z-linked. By contrast, there is clear evidence suggesting strong X- and Z-linkage of hybrid sterility even accounting for the recessivity of this trait (Table 1), and hybrid inviability typically involves interactions between small numbers of X-linked and autosomal loci (Table 1). This means that genes involved in prezygotic isolation are more likely to be the same genes that eventually lead to hybrid sterility and inviability when they are sex-linked, partly because of their presence on the sex chromosomes *per se* and partly due to their likely function in reproduction. Because the X-linked genes in the heterogametic sex have a competitive advantage in the coevolutionary arms race caused by intra-locus sexual conflict (as they are efficiently exposed to positive selection in males whereas they are sheltered from negative selection in females), it is intuitive to envision that recessive X-linked genes determining male reproductive traits diverge quickly between populations. Fast divergence in those traits may, in turn, cause both sexual isolation and sterility in male hybrids. That prezygotic isolation traits are often disproportionately Z-linked is in line with the expectation of an accumulation of a broader range of sexual traits (possibly including female preferences) on this chromosome. Although it is less intuitive to envision that all of these traits will cause hybrid sterility, adaptive speciation is more likely when the male and female component of species recognition can remain in linkage disequilibrium despite periods of gene flow. Thus, according to both theoretical expectations and to preliminary empirical evidence, sex-linked genes have slightly different functions in the speciation process of male- versus female-heterogametic taxa. However, additional empirical information is needed before we can reach a consensus regarding the role of sex-linked genes in male- and female-heterogametic taxa at different stages of the speciation process: are genes coding for certain types of sexual traits more often located on sex chromosomes than others? What is relatively more important for sex linkage—the involvement of few genes in the determination of the trait, recessivity, which sex is heterogametic, or strong sexually antagonistic fitness effects? Does the Z chromosome more often harbour speciation genes than the X-chromosome? What role are epistatic interactions between sex-linked genes and autosomal genes playing at different stages of speciation? Are primary sexual traits more often sex-linked, do they diverge faster and are they more likely to have pleiotropic effects on different facets of reproductive isolation than other traits? The ongoing accumulation of sequence data from different organisms will open up the possibility for answering these types of questions. In particular genomic studies of hybrid zones, which can occur between taxa at all stages of the speciation process, can provide evidence of the genomic distribution of genes under selection (for example, Teeter *et al*., 2008). Such studies can be followed up by more detailed analyses of the specific role in reproductive isolation of the identified genes and genomic regions. This can be followed by comparison of the location of genes causing reproductive isolation across different taxa at different stages of divergence, and with different speciation rates.

Acknowledgements

We thank Hans Ellegren, Judith Mank and Tomas Pärt for helpful discussions and comments on the paper. Dolph Schluter provided the figure legend text for the stickleback photo. This work was financed by a European Young Investigator Award (EURYI, AQ) and by Wenner-Gren Foundations (RB).

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