

Invasion and fixation of sex-reversal genes

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Abstract

We simulated a meta-population with random dispersal among demes but local mating within demes to investigate conditions under which a dominant female-determining gene *W*, with no individual selection advantage, can invade and become fixed in females, changing the population from male to female heterogamety. Starting with one mutant *W* in a single deme, the interaction of sex ratio selection and random genetic drift causes *W* to be fixed among females more often than a comparable neutral mutation with no influence on sex determination, even when YY males have slightly reduced viability. Meta-population structure and interdeme selection can also favour the fixation of *W*. The reverse transition from female to male heterogamety can also occur with higher probability than for a comparable neutral mutation. These results help to explain the involvement of sex-determining genes in the evolution of sex chromosomes and in sexual selection and speciation.

Introduction

The genetics, ecology and evolution of sex determination are a major theme in evolutionary biology (Bull, 1983; Foster *et al.*, 1992; Whitfield *et al.*, 1993; Beukeboom *et al.*, 2000; Hardy, 2002; Schrempf *et al.*, 2006; Zhou *et al.*, 2006). Genetic sex determination can be highly labile in some taxa. In teleost fish, polymorphisms of sex-determining genes within and between closely related populations of the same species can be complex (Lee *et al.*, 2003, 2004) and have been proposed to play a role in the evolution of mating preferences and speciation (Seehausen *et al.*, 1999; Lande *et al.*, 2001; Kocher, 2004). Intrapopulation polymorphisms in sex chromosomes (*W*, *X*, *Y*) were first described in the platyfish (Kallman, 1970; Orzack *et al.*, 1980; Bull, 1983). These chromosomes share most of their genes, are morphologically indistinguishable and probably recombine over most of their length. In this system, chromosome combinations of *WY*, *WX* and *XX* result in female, whereas *XY* and *YY* result in male. *YY* males are possible because *Y* has not yet accumulated significant amounts

of the deleterious mutations common in *Y* chromosome evolution (Charlesworth, 1991, 1996; Liu *et al.*, 2004). Similar systems appear to be widespread in African cichlid fish (Hickling, 1960; Hammerman & Avtalion, 1979; Seehausen *et al.*, 1999; Lande *et al.*, 2001; Lee *et al.*, 2003, 2004; Oldfield, 2005) and appear to be involved in the mechanisms of speciation (Seehausen *et al.*, 1999; Lande *et al.*, 2001).

There is no direct evidence for a selective advantage to individual females bearing the *W* chromosome (Lande *et al.*, 2001) and it is not obvious how dominant sex-reversal genes become established in a population. One possibility is by random genetic drift in a small local population, but this leaves unexplained the problem of spread through a species' geographical range. In the absence of individual selection, sex-ratio selection acts to produce an even (unbiased) sex ratio (Fisher, 1958), and in the case of the *W*, *X*, *Y* chromosome system there is a line of neutral equilibria connecting male heterogamety (*XY* males, *XX* females) to female heterogamety (*YY* males, *WY* females) with an even sex ratio at every point on the line along which populations can drift (Bull, 1983). One possibility to explain spread is interdeme selection in a subdivided meta-population. In a meta-population, local populations (or demes) with a female-biased sex ratio have an elevated productivity

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per capita compared with demes with unbiased sex ratios, and the former will export more emigrants and colonists; demes in which W has very recently increased by random genetic drift are likely to have a female bias, conferring an interdemic selection advantage on W (Hamilton, 1967; Colwell, 1981; Wilson & Colwell, 1981; Lande *et al.*, 2001). Simulations have shown that interdemic selection can overpower individual selection (Wilson & Colwell, 1981) and that biased sex ratios would not evolve in the absence of a group-level selective advantage (Avilés, 1993).

Using a meta-population model with a finite number of demes, we explore conditions favouring the invasion of a dominant female determiner (sex-reversal gene) W in an initially male heterogametic (XY) population, and its fixation (as a heterozygote) in females to establish female heterogamety (WY). The model concerns a male heterogametic species in the early stages of the formation of sex chromosomes, with suppressed recombination in the region of the sex-determining loci, allowing mildly deleterious recessive mutations to begin accumulating on the Y chromosome, which was initially always heterozygous in males (Charlesworth, 1991, 1996). Accordingly, we investigate fixation of W in single populations of different size, with either full viability or slightly reduced viability of YY males. The probability of the reverse transition from female heterogamety (WY) to male heterogametic (XY) is similarly explored. We also investigate the effects of meta-population subdivision and connectivity on the fixation of W. Our model thus treats explicitly the population properties that facilitate the evolution of female-biased sex ratios and the reversal of heterogametic sex, processes relevant for the evolution of sex chromosomes and sex-determining systems (Bull & Charnov, 1977; Charlesworth, 1991, 1996; Wilkins, 1995; Ogata *et al.*, 2003; Mank *et al.*, 2006).

Methods

We used individual-based model simulations to describe the evolution of genotype frequencies in a single population or meta-population when a new mutation introduces a dominant female sex determiner, W. The model is stimulated by observations on African cichlid fish and other teleosts, where dominant female determiners cause genetic sex-reversal, such that XX, WX and WY individuals are female while XY and YY individuals are male (Kallman, 1970; Bull & Charnov, 1977; Orzack *et al.*, 1980; Bull, 1983; Seehausen *et al.*, 1999; Lande *et al.*, 2001; Lee *et al.*, 2004; Mank *et al.*, 2006). We assume a meta-population system with a finite number of demes, n , each containing N individuals. In each deme, the life cycle is the following: (i) viability selection on new zygotes (ii) random dispersal of surviving juveniles among demes, (iii) sampling of adult genotypes and (iv) random mating within demes. The frequency of genotype ij in deme, k , at the beginning of the life cycle,

before viability selection, is denoted as $p_{ij,k}$ where i and $j = X, Y, W$, and $\sum_{ij} p_{ij,k} = 1$. Letting v_{ij} denote the viability of genotype ij , the genotypic frequencies after viability selection are $p_{ij,k}^* = v_{ij} p_{ij,k} / \sum_{ij} v_{ij} p_{ij,k}$. Dispersal occurs similar to Wright's island model. A fraction m of zygotes emigrates from each deme into a migrant pool. Denoting the proportion of adult females in deme k in the previous generation as ϕ_k , the relative contribution of emigrants from deme k to the migrant pool is $mN\phi_k$, such that demes with a higher proportion of females contribute more to the migrant pool. The frequency of genotype ij in the migrant pool in any given generation is given by $Q_{ij} = \sum_k p_{ij,k}^* \phi_k / \sum_k \phi_k$. Dispersal occurs at random (uniformly) across all demes, such that a fraction m of each deme is replaced by immigrants from the migrant pool, $p_{ij,k}^{**} = (1 - m)p_{ij,k}^* + mQ_{ij}$ for all k . Random genetic drift caused by finite population size is modelled as a process of multinomial sampling of N adults from each deme, given the local genotype frequencies after migration in each generation. This yields after multinomial sampling the adult genotype frequencies $p_{ij,k}^{***}$. Finally, the equations for mating and production of zygotes within each deme (suppressing the subscript k for simplicity) are those of Orzack *et al.* (1980):

$$p'_{WY} = (p_{WY}^{***} + p_{WX}^{***})(p_{XY}^{***} + 2p_{YY}^{***}),$$

$$p'_{WX} = p_{XY}^{***}(p_{WY}^{***} + p_{WX}^{***}),$$

$$p'_{XX} = p_{XY}^{***}(p_{WY}^{***} + 2p_{XX}^{***}),$$

$$p'_{XY} = p_{XY}^{***}(p_{WY}^{***} + p_{WX}^{***} + 2p_{XX}^{***}) + p_{YY}^{***}(2p_{WX}^{***} + 4p_{XX}^{***})$$

and

$$p'_{YY} = p_{WY}^{***}(p_{XY}^{***} + 2p_{YY}^{***}).$$

There is no recombination among the sex chromosomes.

The model is thus based on numerical analysis of deterministic and stochastic changes in genotype frequencies in demes. Genotype frequencies after mating and viability selection are determined for a very large (practically infinite) number of juveniles, and stochastic changes appear when a finite number of adults are randomly sampled before mating and reproduction.

Within this framework we simulate the invasion and fixation of a dominant female sex determiner W, by (i) introducing the sex-reversal gene W into the meta-population as one mutant in one deme of an initially male heterogametic population and (ii) starting at an intermediate frequency of W with genotype frequencies in the populations at equilibrium (Orzack *et al.*, 1980) and we vary this initial frequency. Unless otherwise specified, the initial mutation to W arises by changing XX to WX in a population with an initially 1 : 1 sex ratio, but

for comparison we also investigate mutations arising by changing XY to WY. The probability and time of fixation of W is simulated under different scenarios depending on the size of a single population, N ; the degree of subdivision measured by the number of demes, n (holding constant the total meta-population size, nN), and the migration rate, m .

The first simulations concern an unstructured population (one deme) with different sizes $N = 10\text{--}1000$. Secondly, by keeping the meta-population size constant ($nN = 1000$), with panmictic dispersal ($m = 1 - 1/n$) and local mating, we examine the influence of meta-population structure or degree of subdivision ($n = 1\text{--}100$) on the probability of W fixation. Finally, the effect of connectivity is analysed ($m = 0\text{--}0.99$) in a meta-population of defined size and structure (with $n = 10$, $N = 100$). We ran at least 10 000 simulations of each set of parameters until fixation or loss of W and recorded (i) the probability of W being fixed (complete female heterogamety) in the meta-population and (ii) the mean time to fixation.

The dynamics of polymorphisms in sex chromosomes (W, X, Y) under the joint action of random genetic drift and sex-ratio selection are then analysed with the help of a De Finetti diagram. The De Finetti diagram represents in an equilateral triangle the frequencies of the three female genotypes as distances from the edges, with pure populations of the genotypes indicated by labels XX at the lower right vertex, WY at the upper vertex and WX at the lower left vertex (Bull & Charnov, 1977; Bull, 1983). For the case when YY males have no viability disadvantage, we investigated the frequency dynamics around the line of neutral equilibria connecting male heterogamety (all females XX) and female heterogamety (all females WY). This line indicates the complete set of equilibrium frequencies for XX, WX and WY. On this line the two male and the three female genotypes can be obtained by the following formulae (Bull & Charnov, 1977):

$$p_{YY} = p,$$

$$p_{XY} = 1 - p,$$

$$p_{XX} = \frac{(1-p)^2}{(1+p)^2},$$

$$p_{WX} = \frac{2p(1-p)}{(1+p)^2},$$

$$p_{WY} = \frac{2p}{1+p},$$

where p is the YY male genotype frequency.

The impact of interdeme selection on the fixation probability of W in a meta-population was revealed by comparing simulations with or without interdeme selection. Elimination of interdeme selection was accomplished by equalizing the contribution of all demes to the migrant pool, regardless of the parent sex ratio in the demes. The reverse transition from female heterogamety to male heterogamety also was investigated by introducing a single mutant X chromosome (changing WY to XY) in one individual in a single deme, with or without a viability disadvantage to YY males.

Results

Population size

With no individual selection advantage for W, the interaction of random genetic drift and sex ratio selection in a single deme can facilitate the fixation of W, reversing the heterogametic sex. Starting from a single mutant, the probability of fixation of W decreases with increasing population size (Fig. 1a). Starting at different frequencies, the probability of fixation of W increases with the initial frequency of W (Fig. 2a). Frequency dependence of the process is shown by the departure of Fig. 2a from linearity, indicating an advantage at low frequency and a disadvantage at high frequency caused by the interaction of random genetic drift and sex ratio selection. The results do not change appreciably if W arises by mutation of X to W in either of the genotypes XX or XY (Fig. 1a). Starting from a single copy, the mean time to fixation of W in a single deme is about 700 generations, nearly independent of deme size (Fig. 1d); starting from different frequencies of W, the mean time to fixation decreases with increasing initial frequency in the population (Fig. 2b).

Comparison with the probability of fixation of a neutral gene with no influence on sex determination (Fig. 3) also shows that W has a frequency-dependent advantage, which decreases with increasing initial frequency of W. The interaction of sex ratio selection and random genetic drift tends to strongly increase the relative fixation probability of W at low initial frequencies, reaching a relative probability of 20 for a population size of 1000 (Fig. 3a). Even with slightly reduced viability of YY males, in both a single population and in a meta-population, the probability of fixation of W remains much larger than for a comparable neutral mutation. An intuitive explanation for this advantage at low frequencies, in the case when YY males have unreduced viability, is provided by examining the dynamics of female genotype frequencies around the line of neutral equilibria in the De Finetti diagram (Fig. 4). Every point on this line has a sex ratio of 1 : 1 [also including male genotype frequencies (XY, YY) in two more dimensions not shown in the graph]. In this diagram we plot deterministic evolutionary trajectories (in an infinite

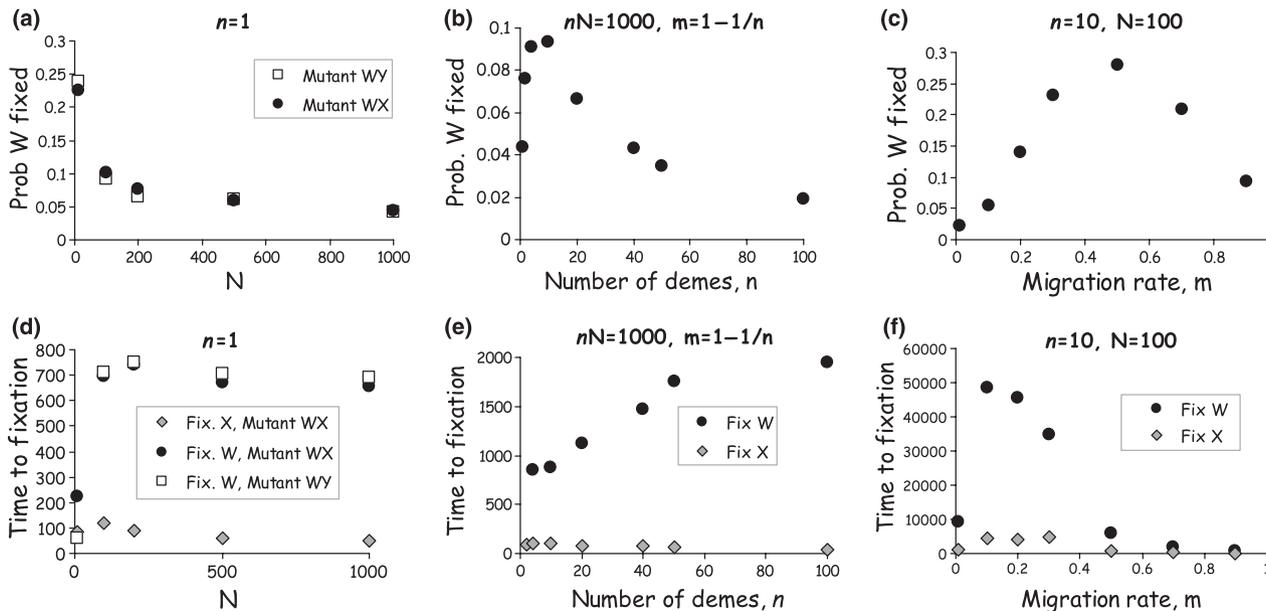


Fig. 1 The probability of fixation of W (achieving complete female heterogamy) starting with a single mutant in a single deme, as a function of (a) the size of a single isolated deme, N , (b) meta-population subdivision indicated by number of demes, n , with $nN = \text{constant}$ and panmictic dispersal, $m = 1 - 1/n$, and (c) migration rate among demes, m . Mean times to complete fixation of W are shown for each case respectively (d), (e) and (f).

population) resulting from random mating and sex-ratio selection, starting from both sides of the line of equilibria. (In a finite natural population, random genetic drift will cause the population to fluctuate around both sides of the line of equilibria.) Near the lower right corner, where W is initially rare, the evolutionary trajectories are nearly horizontal on either side of the line of equilibria. With a low initial frequency of W on the line of equilibria, in populations that drift above the line of equilibria W will tend to increase, and in populations below the line W will tend to decrease. Although a finite population is likely to fluctuate on both sides of the line of equilibria, some trajectories will be accelerated towards XX (loss of W), whereas others will be accelerated towards WY (fixation of W). This indicates that the interaction of random genetic drift with sex-ratio selection makes populations diverge faster than random genetic drift alone. Because of asymmetry of the possible scope for increasing vs. decreasing the frequency of W when it is rare, this interaction produces the net result of increasing the probability of fixation of a new W mutation beyond that which would occur by random genetic drift alone.

The reverse transition from female to male heterogamy also occurs with higher probability than for a neutral mutation, although not quite as high as for the transition from male to female heterogamy (Fig. 5). When YY males have unreduced viability, the inequality between the probabilities of forward and backward transitions in heterogametic sex can be explained by

asymmetry of the sex-determining system (with W dominant to Y and Y dominant to X) and the corresponding asymmetry in the line of neutral equilibria in the De Finetti diagram (Fig. 4). The interaction of random genetic drift with sex ratio selection occurs differently in the crucial initial stages when a W mutation invades a male heterogametic population compared with when an X mutation invades a female heterogametic population. Reduced viability of YY males reduces the probability of transition from male to female heterogamy, and favours the reverse transition (Fig. 5).

Meta-population structure

The meta-population model with panmictic dispersal and local mating shows that the probability of fixation of W is maximized by an intermediate degree of subdivision (Fig. 1b,e). Meta-population structure creates variance in sex ratio among demes causing interdeme selection by differential contribution among demes to the migrant pool. The absolute probability of fixation of a single mutant can be remarkably high, up to about 10% with moderate subdivision in a total meta-population of 1000 individuals, whereas the fixation probability is about 5% when the population consists of a single deme of 1000 individuals. It therefore appears that no individual selection advantage is necessary to explain invasion of a meta-population by W, as this is likely to happen spontaneously with a substantial probability after the

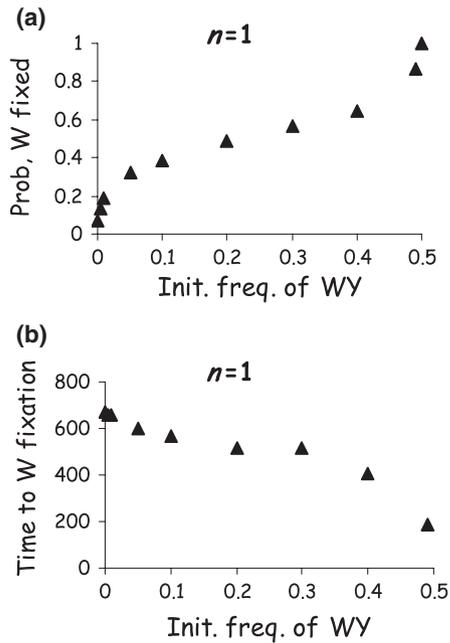


Fig. 2 Probability (a) and mean time (b) for W to be fixed in a single isolated deme, starting with various initial frequencies of W and equilibrium genotype frequencies (Orzack *et al.*, 1980): $p_{WY} = 2p_{WY}/(1/2 - p_{WY})$, $p_{XX} = 2(1/2 - p_{WY})^2$, $p_{XY} = (1/2 - p_{WY})(1 - p_{WY})$, and $p_{YY} = p_{WY}/2(1 - p_{WY})$.

mutation has appeared. Here again simulations showed that, even with slightly reduced viability of YY males, W has a much higher probability of fixation than a neutral gene. Again, under panmictic dispersal with local mating the probability of fixation of W is maximized by an intermediate degree of subdivision of the meta-population (Fig. 3b).

Meta-population connectivity

In the meta-population model comparing different migration rates among demes, we find that fairly high dispersal rates, around $m = 0.4-0.5$, yield the highest probability of fixation of a new W mutant (Fig. 1c). Thus strong isolation between demes (small m) is not necessary and even hinders the fixation of W. The fixation of W could likely also happen efficiently in a continuously distributed population with limited dispersal (isolation by distance). The mean number of generations until fixation of W in the entire meta-population is negatively correlated with migration rate and can reach 50 000 generations at low migration rates (Fig. 1f).

Interdeme selection

Comparing meta-populations with and without interdeme selection (Fig. 6), assuming panmictic dispersal and

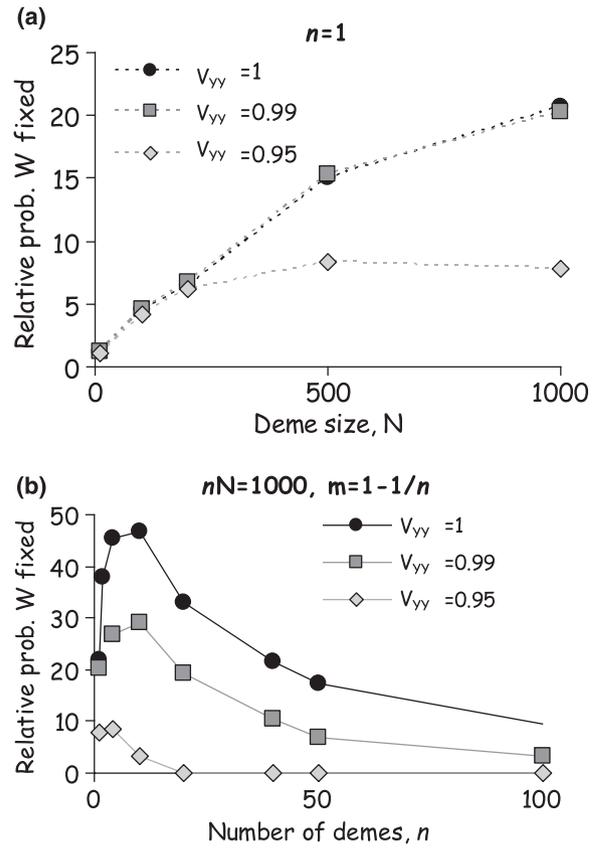


Fig. 3 Relative probability of W being fixed starting from a single mutation. This is the probability of fixation divided by the initial frequency in females, $1/(N/2)$, in a single isolated deme (a) or $1/(nN/2)$ in a meta-population with n demes (b). The comparison is to a neutral haploid mutation, or a neutral mutation on a dominant sex-determining chromosome, inherited only through one sex, of which there are $N/2$ in a deme with an initially even sex ratio. Results are illustrated for different values of the viability of YY males, v_{yy} .

local mating, we find that interdeme selection can substantially augment the fixation probability of W. This occurs especially at an intermediate degree of subdivision that favours the transition to female heterogamety.

Discussion

Bull & Charnov (1977) hypothesized that a new sex-determining gene can rapidly increase and become fixed in a population if it is linked to a gene with high adaptive value, and finally cause a change of the heterogametic sex. Here we have demonstrated that linkage to a gene with a selective advantage is not required for a new dominant female determiner to become fixed in a population or meta-population. Our simulations suggest that the interaction of genetic drift and sex-ratio selection causes the sex-determining gene W to invade and become fixed in females, reversing the heterogametic

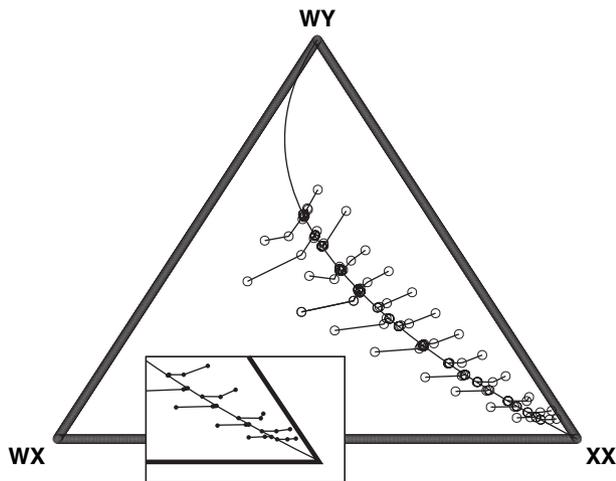


Fig. 4 De Finetti diagram describing deterministic dynamics of female genotype frequencies around the line of neutral equilibria. The frequency of a genotype in females is given by the perpendicular distance from the point to the axis opposite the vertex labelled with that genotype, and the three frequencies necessarily add to unity. The solid curve indicates the complete set of equilibrium frequencies for XX, WX and WY, connecting the two endpoints of male and female heterogamety. The equilibrium frequencies of the two male genotypes XY and YY can be obtained by the following formulae (Bull & Charnov, 1977): $p_{YY} = p$, $p_{XY} = 1 - p$, $p_{XX} = (1 - p)^2 / (1 + p)^2$, $p_{WX} = 2p(1 - p) / (1 + p)^2$, $p_{WY} = 2p / (1 + p)$. Evolutionary trajectories starting from both sides of line of equilibria are followed for 10 generations.

sex, with higher probability than a comparable neutral mutation with no sex-determining effect. Interestingly, this happens both at population and meta-population levels without any individual selection advantage. The process is frequency-dependent, and is optimized by an intermediate degree of meta-population subdivision with fairly high dispersal rates among demes.

We have shown that within a single isolated deme the sex-reversal gene W has a substantially higher probability of fixation than a neutral gene (Fig. 3). The interaction of random genetic drift with sex-ratio selection makes populations diverge faster than by random genetic drift alone and increases the probability of fixation of a new W mutation within a single local population. As shown by the De Finetti diagram (Fig. 4) describing deterministic evolutionary trajectories around the line of equilibria, this interaction tends to increase the frequency of W when it is initially low.

Interdeme selection favours demes with female-biased sex ratio because of their higher productivity compared with demes with unbiased sex ratios (Colwell, 1981; Wilson & Colwell, 1981; Charnov, 1982; Avilés, 1993). As shown by our simulations, interdeme selection can substantially increase the probability that W becomes fixed (Fig. 6). There is no need for strong isolation or subdivision between demes (Fig. 1c), although an inter-

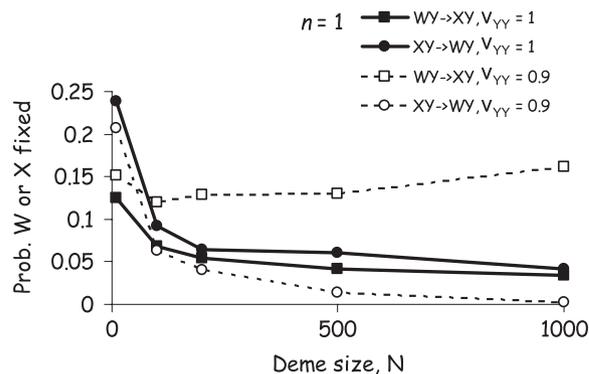


Fig. 5 Relative probability of fixation of W starting from a single mutation in a male heterogametic population (XY males) (as in Fig. 3), and relative probability of fixation of X starting from a single mutation in a female heterogametic population (WY females), in a single isolated deme.

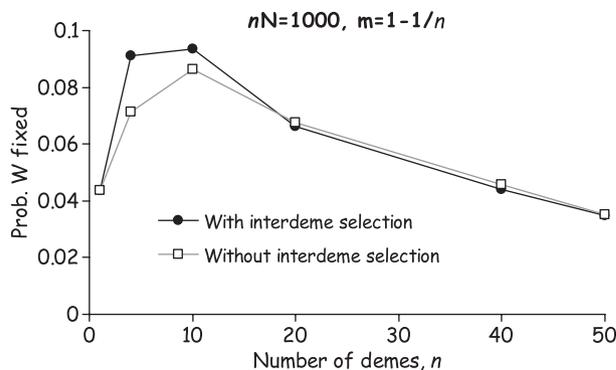


Fig. 6 Probability of fixation of W starting from a single mutation in a single deme, in a meta-population with panmictic dispersal and local mating, with or without interdeme selection. (For 20 or more demes, the fixation probabilities with and without interdeme selection differ by less than the sum of their standard errors based on 40 000 simulations.)

mediate degree of subdivision (for fixed nN) facilitates fixation of W by magnifying random genetic drift and increasing the variance in sex ratio among demes, conferring a greater interdeme selection advantage on W. It is therefore likely that similar processes can facilitate fixation of W in geographically distributed populations with local dispersal (isolation-by-distance). The number of generations that W segregates on the way to fixation increases with greater meta-population substructure and decreases with larger migration rate (Fig. 1e,f).

Polymorphism for sex-determining genes within or among populations has been reported in many species including houseflies, midges, woodlice, platyfish, cichlid fish, and frogs (Gordon, 1944; Kallman, 1970; Thompson, 1971; Macdonald, 1978; Bull, 1983; Rigaud *et al.*,

1997; Caubet *et al.*, 2000; Lande *et al.*, 2001; Ogata *et al.*, 2003; Lee *et al.*, 2004; Mank *et al.*, 2006). Our model shows that invasion of a local population or a meta-population by a dominant female-determining gene, and its fixation in females, is feasible without any individual advantage to the new sex-determiner. This can occur under a wide range of meta-population structure, with no or moderate subdivision and fairly high migration rates, even with slightly reduced viability of YY males. Remarkably, the probability of both forward and backward transitions between male and female heterogamety may be much higher than for selectively neutral mutations (Fig. 5). This supports the suggestion that male and female heterogamety may switch back and forth repeatedly within a lineage over evolutionary time (Wilkins, 1995; Lande *et al.*, 2001; Mank *et al.*, 2006). Such dynamic evolution of sex-determining genes may provide opportunities for their interaction with polymorphisms involved in mate choice, speciation, and chromosome evolution as proposed by Seehausen *et al.* (1999), Lande *et al.* (2001) and Mank *et al.* (2006). This hypothesis should be thoroughly tested in future, e.g. through comparative phylogenetic analyses that are currently still data-limited.

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