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Maintaining heritable variation via sex-limited temporally fluctuating selection: a phenotypic model accommodating non-Mendelian epigenetic effects

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Summary: Using a phenotypic model, we show that significant heritable variation can be maintained in a population subjected to temporally fluctuating selection if only one sex is subject to selection. In fact, more variation is maintained with sex-limited selection at a given selection intensity than if both sexes are subject to half that selection intensity. This result is commensurate with existing population genetic models. However, genetic models may be inappropriate for sexually selected traits because many of them may be of non-genetic origin, such as maternal effects or – more likely – epigenetic effects. Phenotypic models obviate this problem by accommodating both genetic and epigenetic effects, as well as maternal effects. Our phenotypic model of sex-limited temporally fluctuating selection shows that substantial heritable variation can be maintained and thereby provides impetus to develop population epigenetic models.

Introduction

Minimal heritable variance in either fitness or its components has become a long-held expectation, based on the notion that fitness is usually influenced by directional selection, and therefore a single best genotype should predominate (Fisher 1930; Falconer 1981; Charlesworth 1984; Charlesworth 1987). However, fitness-conferring traits typically exhibit higher levels of variation than other traits (Houle 1992), and sexual characters exhibit even higher levels of variation than other fitness-conferring traits in similar taxa (Pomiankowski and Møller 1995). The inconsistencies between theory and data reveal the importance of determining the mechan-

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isms responsible for maintaining heritable variation in sexually selected traits.

Present evolutionary theory indicates temporally fluctuating selection is too restrictive to maintain genetic variation in a population, unless there is heterozygote advantage (Hedrick 1986) or generation overlap provides a 'storage effect' for less advantageous genotypes to survive until the next favorable selective event occurs (Ellner and Hairston 1994; Sasaki and Ellner 1995; Ellner 1996; Ellner and Sasaki 1996; Sasaki and Ellner 1997; Ellner et al. 1999; Sasaki and de Jong 1999). Variation maintenance is, however, expected in a population exposed to temporally fluctuating selection provided segments of the population are exposed to different selective regimes; sex-limited selection provides such an example (Sasaki and Ellner 1997).

Following Sasaki and Ellner (1997), we propose that sex-limited temporally fluctuating selection can maintain heritable variation within a population. Our hypothesis is depicted as follows. When a trait is expressed in only one sex, selection on that trait can only affect the reproductive success and survival of that sex. When males, for example, are the only sex to produce a mating signal, loci coding for the trait carried by females would be unexpressed and therefore hidden from selection within a single generation. Some proportion of the alleles or other heritable epigenetic signals coding for sex-limited traits would therefore be shielded from selection. Through mating and chromosomal segregation, subsets of less advantageous genes or epigenes could be transferred from mothers into offspring (Bertram 1999; Reinhold 1999; Reinhold 2000). In subsequent generations some males would display phenotypes that, although disadvantageous in the previous generation, would now be favored by selection. Higher frequencies of unfavorable genes or epigenetic signals would therefore remain in the population than if selection had not been sex-limited.

A handful of previously developed models indicate that sex-limited selection may be an important mechanism in the maintenance of heritable variation in sex-limited traits (Owens 1953; Haldane 1962; Li 1963; Kidwell et al. 1977). Most, however, explain maintenance of heritable variation in conjunction with heterozygote advantage, and without temporal fluctuations in selection. Two models provide support for the hypothesis that sex-limited temporally fluctuating selection can maintain genetic variation without the inclusion of heterozygote advantage. The first indicates that sex-limited temporally fluctuating selection can result in rare alleles invading a population under an array of initial conditions (Reinhold 1999). The second shows temporally fluctuating selection can maintain a genetic polymorphism in sex-limited traits whenever the selection regime is symmetrical, where selection switches between positive and negative selection each generation, and in many cases when nonsymmetrical (Reinhold 2000). While these models are suggestive of fluctuating selection's influence on genetic variation, they assume sex-limited trait expression is influenced by

only two alleles at one locus. Although this assumption is simplifying, most heritable sex-limited traits are likely to be influenced by genes at many loci, maternal or paternal effects (which we refer to as parent-of-origin effects), or, may be derived from heritable differentially methylated regions, i. e. epigenetic effects.

Although many heritable sexually selected traits are commonly considered genetic in nature, epigenetics is an equally likely explanation. Evidence suggests that heritable phenotypic variation in some organisms is due to variation in DNA methylation levels, not variation in nucleotide sequences (Van Speybroeck 2000). Flower shape in butter-and-eggs *(Linaria vulgaris,* Scrophulariaceae) is under epigenetic control (Cubas et al. 1999). Sex in several lineages appears to be determined by genomic imprinting (Crouse 1960; Chandra 1985; Marec and Novák 1998; Gorelick and Osborne 2002; Gorelick 2003). Sex-biased dispersal (Haig 2000) and maternal behaviors (Lefebvre et al. 1998) of certain mammals and birds are caused by genomic imprinting. Size of the portion of the zebra finch brain that controls singing (Gahr and Metzdorf 1999; Airey et al. 2000) is an epigenetic effect that likely operates by steroidal hormones changing heritable methylation patterns (McLachlan et al. 1998). More tenuously, genomic imprinting is believed to be the cause of some cognitive abilities (Isles and Wilkinson 2000) and even alcoholism in mammals (Chorney et al. 1998).

Sex-limited traits are traditionally thought to be inherited through Mendelian genetic mechanisms. However, there are several other molecular mechanisms that allow sex-limited traits to be heritable. In the following three paragraphs, we describe these molecular mechanisms in greater detail and argue whether they provide common or rare causes for sex-limited traits.

Sex-limited traits could be coded by genes on sex chromosomes. However, heterogametic sex chromosomes carry few genes (Charlesworth 1991), and instances where they harbor genes coding for sexually selected traits are extremely rare. In fact, approximately two-thirds of genes coding for sexually selected traits reside on autosomes, while in the other third of the cases, they lie almost exclusively on the X-chromosomes (Reinhold 1998). For example, genomic imprints are usually fairly evenly distributed across the genome, except are rarer on Y-chromosomes because of their (often) reduced chromosome size. Thus, most genetic and epigenetic causes for sex-limited traits are not likely to reside on sex chromosomes.

Reinhold (1999) argues that mitochondrial and other parent-of-origin effects are not likely to play a strong role in influencing sex-limited characters. However, it seems that in a few lineages such as bivalves, sex-limited mitochondrial effects could potentially be large (Zouros et al. 1994; Hoeh et al. 1996; Stewart et al. 1996; Passamonti and Scali 2001). In bivalves with so-called doubly uniparental inheritance (DUI), females only inherit mitochondria from their female parent, whereas males inherit mitochondria from both female and male parents. Thus, one sex is (relatively speaking)

shielded from selection on mitochondrial genomes. But, because we are using a phenotypic model, these details do not matter; such parent-of-origin effects are implicitly subsumed in our model. Sex differences in organelle inheritance are highly unusual, only being found in bivalves and conifers (Mogensen 1996; Passamonti and Scali 2001; Whittle and Johnston 2002), hence we ignore them throughout much of the remainder of this paper.

The most prominent molecular form of a sex-limited trait is genomic imprinting. With genomic imprinting, each sex contains their own unique epigenetic pattern, often in the form of methylation patterns, but also in the form of proteins bound to the DNA. It has even been argued that female and male patterns of methylation are complements (i. e. virtually mirror images) of one another (Pardo-Manuel de Villena et al. 2000). Since epigenetic patterns, especially of promoter regions, are known to regulate genes, we argue that it is likely that genomic imprints are likely source for many sex-limited traits.

Our goal is to explore the basic conditions under which heritable variation can be maintained in a sexually selected trait via sex-limited temporally fluctuating selection. To do this, we develop a phenotypic simulation model. We justify using a phenotypic model because it is believed to be comparable to more standard genetic models (Eshel and Feldman 1982; Eshel et al. 1998; Cheptou and Mathias 2001), it can provide added insight into the problem, and most importantly, it allows us to incorporate genetic, parent-of-origin, and especially epigenetic phenomenon. There has been a recent resurgence in modeling phenotypic evolution in terms of epigenetics (Schlichting and Pigliucci 1998; Müller and Newman 1999; Wolf et al. 2001). Modeling of epigenetic phenomena is largely handled as a black box, such as via quantitative genetics (Whitelaw and Martin 2001), despite substantial recent inroads into explicating the molecular basis for many epigenetic phenomena (Russo et al. 1996). By building a phenotypic model, we too follow a black box approach, although we are actively pursuing more reductionist population epigenetic models.

Model Assumptions

As reviewed above, we assume that any genes or epigenes coding for sexually selected traits are not located on the Y-chromosomes (assuming males to be the heterogametic sex), and that mitochondrial and parent-of-origin effects are minimal.

We also assume an effectively infinite population with no heterozygote advantage, discrete (non-overlapping) generations, where genotypes and epigenotypes map directly onto phenotypes. That is, we assume a one-to-one correspondence between the phenotype and the genotype or epigenotype, with no environmental contributions, no genotype-environment interac-

tion, and no epigenotype-environment interaction. Each individual is therefore specified only by its phenotype, the phenotypic value of which is drawn from a discrete finite set of whole numbers from 1 to N. Because population sizes are infinite, we can assume without loss of generality that there are equal numbers of females and males in each generation, despite any sex-limited selection.

Because this is a phenotypic model, we do not specify the number of loci, alleles, or epigenetic marks per locus. The effects of dominance, epistasis, and pleiotropy are not explicitly modeled.

Model Description

Our phenotypic model is an iterative sequence of three subroutines that run in succession each generation: (1) selection, (2) mating and inheritance, and (3) mutation (Fig. 1). For each generation, we first impose selection. The fitness function, which is described in greater detail below, incorporates both sexual and natural selection and can be different for females and males. Since sexual selection is incorporated directly into our fitness function, all individuals that survive selection will mate. All mating individuals

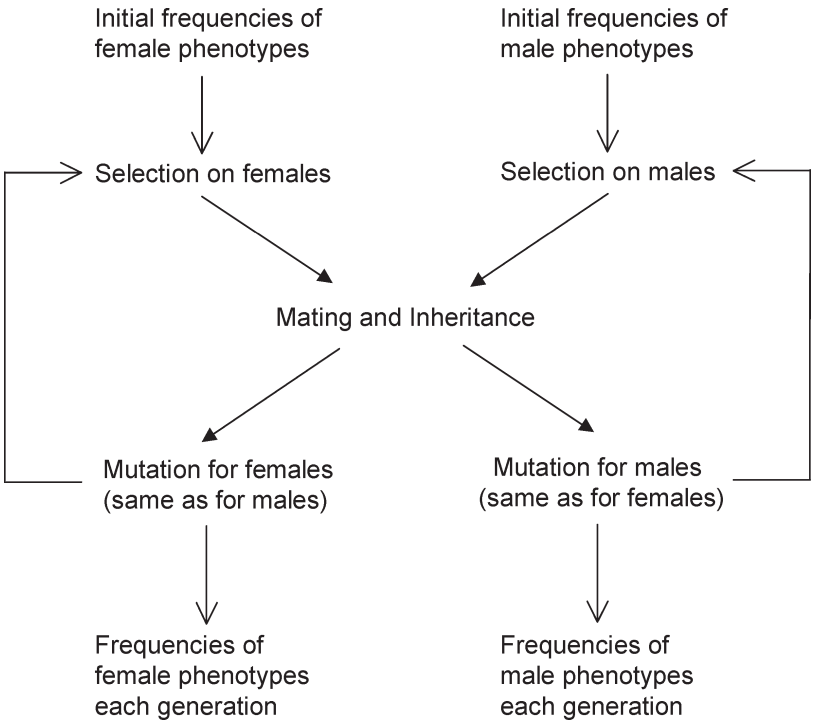

Fig. 1. Schematic of the iterative phenotypic model.

produce an equal number of progeny. The infinite populations of females and males mate either randomly or assortatively, depending on which simulation we run. For assortative mating, individuals mate only if their phenotypes are identical and if there is a large enough proportion of males with that phenotype for the females to find mates (the 'sufficient proportion of males' is a model parameter). Half of all offspring are female and half are male. Because we specify each class of individuals by a single number – its phenotype – we could not model meiotic segregation. Progeny therefore inherit with equal probability the genotype/epigenotype of one of their two parents, thereby precluding blending inheritance. All offspring are subjected to a single round of mutation and then become parents for the subsequent generation.

We incorporate mutation in a different manner than traditional fluctuating selection models. We specify the progeny's mutation or epimutation rate *a priori* and, if a mutation, epimutation, or heritable change in a parent-of-origin effect occurred, then we draw the resulting phenotype from a truncated (censured) normal distribution whose mean is centered at the original phenotypic value of the parent. This technique of incorporating mutations and epimutations (and parent-of-origin effects) is akin to additive genetic models because mutations are more likely to cause small phenotypic effects than large ones. For epimutations, this assumption is supported by the observation that the higher the number of methylated nucleotides at a gene, the greater the suppression of transcription (Razin and Cedar 1991; Boyes and Bird 1992) and the larger the impact on DNA structure (Derreumaux et al. 2001). Mutation and epimutation were assumed to have very small effects, altering only 0.0001 of the genotypes or epigenotypes.

We modeled directional selection with an arctangent function, to allow flexibility in selection's intensity. (I; Fig. 2a) and shape (ε; Fig. 2b). Because phenotypes. (Y) are indexed. ($i = 1, 2, \ldots, N$) the resulting fitness (w) is a weakly monotone function of Y_i, such that (1) $w(Y_1) = {}^1/_2 - {}^1/_2 I$, (2) $w(Y_N) = {}^1/_2 - {}^1/_2 I$, (3) $w(-\infty) = {}^1/_2 - {}^1/_2 I - \varepsilon$, and (4) $w(\infty) = {}^1/_2 + {}^1/_2 I + \varepsilon$. Using these four constraints, the following fitness equation can be solved for its four coefficients

$$w(Y_i; A, B, C, D) = A + B \arctan (Y_i C + D),$$

where the coefficients A, B, C, and D depend only on the parameters $I \in [-1, +1]$ and ε($\varepsilon \approx 0$ yields a step function; $0.05 < \varepsilon < 0.8$ yields a sigmoidal function; $\varepsilon > 1$ yields an approximately linear function).

Statistical Analyses

We established how robust results were to changes in the intensity and shape of selection by running our model under the range of possible para-

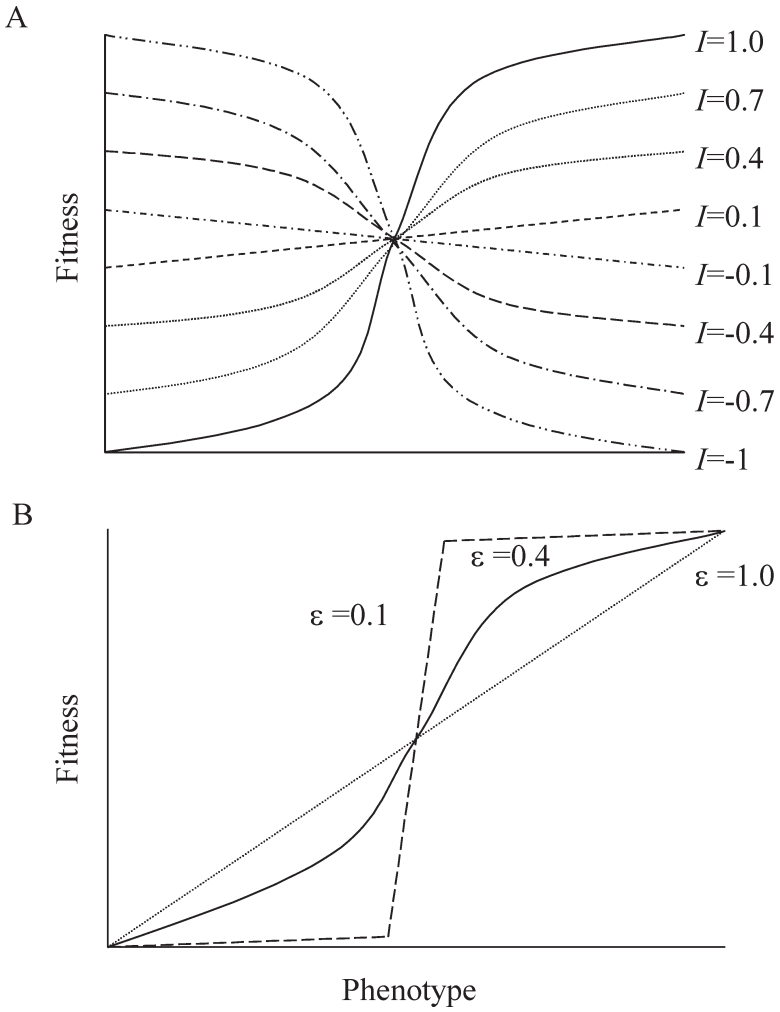

Fig. 2. How fluctuations in selection intensity (I) and shape (ε) influence the selection function. (A) the influence of changing I from –1 to +1. Negative intensities represent a negative relationship between phenotype and fitness, while positive intensities represent a positive relationship. The closer the values are to ±1, the stronger the intensity, the closer to zero, the weaker the intensity; (B) the influence of changing ε from 0 to 1. Zero indicates a step function, 0.5 a sigmoidal function, and 1.0 a linear function.

meters of both intensity and shape. When examining shape's influence, we held the intensity constant at $I = \pm 0.5$, and examined equilibrium results under different ε set points between 0 and 1. When examining intensity's influence, we held the shape constant at $\varepsilon = 0.5$, and examined equilibrium results under different I set points between 0 and ±1. We used ANOVA to determine if either selection intensity or shape influenced the amount of variation maintained in the population at equilibrium. For these analyses, the selection regime was symmetrical and always averaged to zero; every generation the intensity changed between negative and positive values

(i. e., for $I = \pm 0.5$, six generations of selection looked like $I = 0.5, -0.5, 0.5, -0.5, 0.5, -0.5$).

A symmetrical selection regime is thought to be able to maintain the most variation in a population (Reinhold 1999). To establish the result's robustness when the pattern of selection was asymmetrical (i. e., selection changes through time while maintaining a zero mean), we increased the number of generations (G) required before selection switched from positive values to a negative value. We held the shape of the selection regime constant at $\varepsilon = 0.5$, and examined equilibrium results under $I = -0.9$ and $0.9/(G - 1)$. For example, when $G = 3$, six generations of selection looked like ($I = 0.45, 0.45, -0.9, 0.45, 0.45, -0.9$). When $G = 10$, twenty generations of selection looked like ($I = 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, -0.9, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, -0.9$). We used ANOVA to determine if an asymmetrical pattern of selection influenced the amount of variation maintained at equilibrium in the population.

To determine whether sex-limited selection regimes maintain more variation than selection influencing both sexes, we used ANOVA to compare the scenarios of temporally fluctuating selection influencing only males (sex-limited selection) with that of it influencing both sexes (selection on both sexes). We used two basic measures of variation, (1) the proportion of phenotypes remaining in the population at equilibrium (note that a phenotype has to have a proportion of at least 0.001 to be counted by this method), and (2) the Shannon Weaver index ($SW = -\Sigma p_i \ln(p_i)$; Shannon and Weaver 1949) a ubiquitous measure of variation used in numerous disciplines from mathematics to electrical engineering to conservation biology to physics. Because our model was phenotypically based, we could not use conventional measures of variation such as heterozygosity. However, even if multi-locus models of genomic imprinting existed, it would still probably be necessary to use our phenotypic measures of diversity because methylation patterns are much more dynamic than are nucleotide sequences (Gorelick In press). Heritable methylation patterns can change during the course of development (Holliday and Pugh 1975), change when exposed to hormones (LoSchiavo et al. 1989; Jost and Saluz 1993; McLachlan et al. 1998), and even change when exposed to environmental shocks (Demeulemeester et al. 1999; Tatra et al. 2000). Furthermore, Shannon Weaver indices seem to be a natural choice when focusing on parent-of-origin effects that are entirely phenotypically based.

Because our statistical analyses resulted in our running seven different ANOVAs, we Bonferroni adjusted the acceptable values to $p < 0.00714$.

Results

Sex-limited temporally fluctuating selection resulted in significantly more intra-population variation being maintained than selection acting on both

sexes under all selection intensities (Fig. 3a, b). Further, while the number of phenotypes maintained by sex-limited selection dropped with increasing selection intensity, at least 20 % of the phenotypes were maintained even under high selection intensities (Fig. 3b; ANOVA, Shannon Weaver

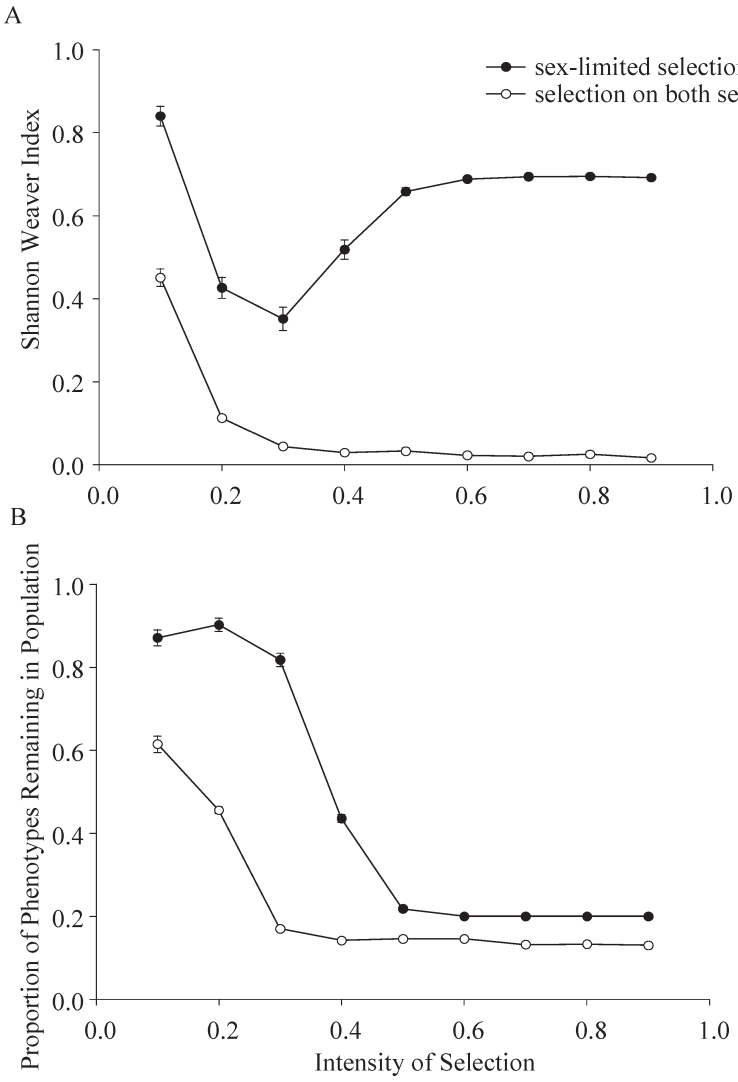

Fig. 3. Model output showing the influence of selection intensity (I) on the amount of variation maintained in the population under two temporally fluctuating selection scenarios: sex-limited selection and selection influencing both sexes. Selection is symmetrical: for each point on the graph, the selection intensity was specified, and then changed from a positive value to a negative value every generation to indicate selection's changing direction. The selection shape, ε, was kept constant at 0.05, as was the mutation rate at 0.0001 mutant genotypes per generation. Points represent the average variation maintained at equilibrium ($N = 100$ runs); error bars indicate the standard error. (A) Shannon Weaver measure of variation remaining in the population; (B) Proportion of phenotypes remaining in the population.

Index – $R^2 = 0.913$, df = 1782, Selection Intensity: $F = 169.0$, $P < 0.001$, Selection Type: $F = 7061.8$, $P < 0.001$, Type x Intensity Interaction: $F = 70.8$, $P<0.001$; Proportion of Phenotypes Remaining – $R^2 = 0.943$, df = 1782, Selection Intensity: $F = 1245.7$, $P < 0.001$, Selection Type: $F = 2414.9$, $P < 0.001$, Type x Intensity Interaction: $F = 250.2$, $P < 0.001$).

While sex-limited temporally fluctuating selection always resulted in significantly more intra-population variation maintenance than selection acting on both sexes under all shape parameters, the shape of the selection function did not significantly influence the amount of variation maintained at equilibrium in the Shannon Weaver analysis. (ANOVA, Shannon Weaver – $R^2 = 0.689$, df = 1782, Selection Shape: $F = 2.3$, $P = 0.016$, Selection Type: $F = 1577.5$, $P < 0.001$, Type x Shape Interaction: $F = 1.5$, $P = 0.157$). However, in the proportion of phenotypes remaining in the population analysis, the shape of selection significantly influenced the population variation. Step-like selection functions maintained greater population variation than graded changes in selection (ANOVA, Proportion of Phenotypes – $R^2 = 0.948$, df = 1782, Selection Shape: $F = 279.1$, $P < 0.001$, Selection Type: $F = 12961.9$, $P < 0.001$, Type x Shape Interaction: $F = 70.2$, $P < 0.001$).

Above results were based on a symmetrical selection regime where selection fluctuated every generation between positive and negative values with a mean of zero, an ideal scenario for variation maintenance (Reinhold 2000). When selection is instead asymmetrical, fluctuating every third, tenth, or even every hundredth generation, sex-limited selection still results in the maintenance of more variation than selection acting on both sexes under most selection regimes (Fig. 4a, b; ANOVA, Shannon Weaver – $R^2 = 0.519$, df = 2376, Selection Pattern: $F = 43.3$, $P < 0.001$, Selection Type: $F = 186.9$, $P < 0.001$, Type x Pattern Interaction: $F = 19.3$, $P < 0.001$; Proportion of Phenotypes – $R^2 = 0.741$, df = 2376, Selection Pattern: $F = 219.0$, $P < 0.001$, Selection Type: $F = 385.4$, $P < 0.001$, Type x Pattern Interaction: $F = 8.5$, $P < 0.001$). The amount of variation maintained in the population is substantially higher when selection is strong and changes direction frequently (every second, third or fourth generation), or when selection is weak and seldom changes direction (weak positive selection for twenty or more generations before a generation experiences strong negative selection). When the asymmetrical sex-limited selection regime is instead moderate in strength and changes direction every 5–20 generations, very little heritable variation remains in the population.

We use the Shannon Weaver index as a proxy for heritable variation. Therefore, when temporally–fluctuating selection acts on both sexes, we expect a diminution in the Shannon Weaver index and an increase in its reciprocal. However, if sex-limited temporally-fluctuating selection maintains heritable variation, we expect that the reciprocal of the Shannon Weaver index will either drop or remain relatively constant as the intensity

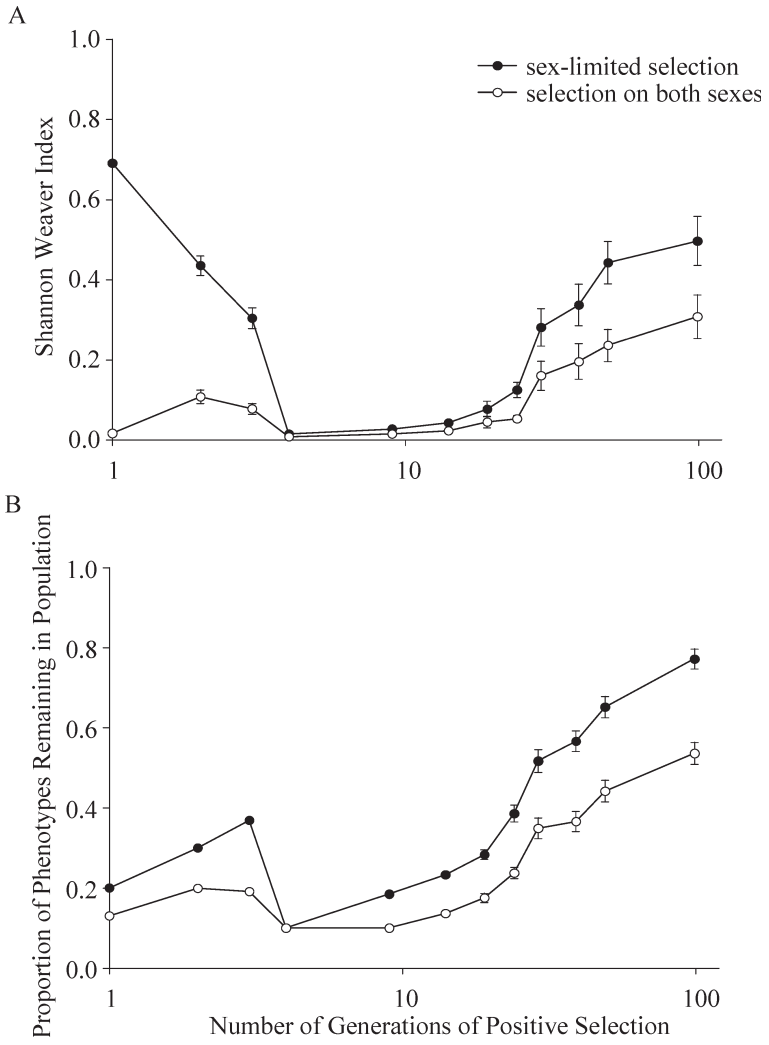

Fig. 4. Model output showing the influence of the pattern of asymmetric selection on the amount of variation maintained in the population under two temporally fluctuating selection scenarios: sex-limited selection and selection influencing both sexes. For each point on the graph, selection intensity was positive for one to several generations, then switched to a negative value of –0.9 for one generation, before switching back to positive values again. Because selection intensity maintained a zero mean, the positive values of selection intensity were dependent upon the number of generations (G) of positive selection, such that $I = 0.9/(G - 1)$. Selection shape was held constant at $\varepsilon = 0$, mutation rate at 0.0001 mutant genotypes per generation. Points represent the average variation maintained at equilibrium (N = 100 runs); error bars indicate standard error. (A) Shannon Weaver measure of variation remaining in the population; (B) Proportion of phenotypes remaining in the population.

of directional selection increases. Using the reciprocal of the Shannon Weaver index allowed us to run a linear regression, rather than cope with a non-linear regression of the raw Shannon Weaver index versus selection intensity. Similar to genetic models, when selection influenced both sexes,

we found that variation was maintained at equilibrium only under extremely low intensity selection regimes. The amount of heritable variation maintained in the population dropped as selection intensity increased (Fig. 5A; Regression: $t = 32.6$, $F = 1062.5$, $P < 0.001$, $R^2 = 0.736$, $df = 898$). In contrast, the amount of heritable variation maintained in the population

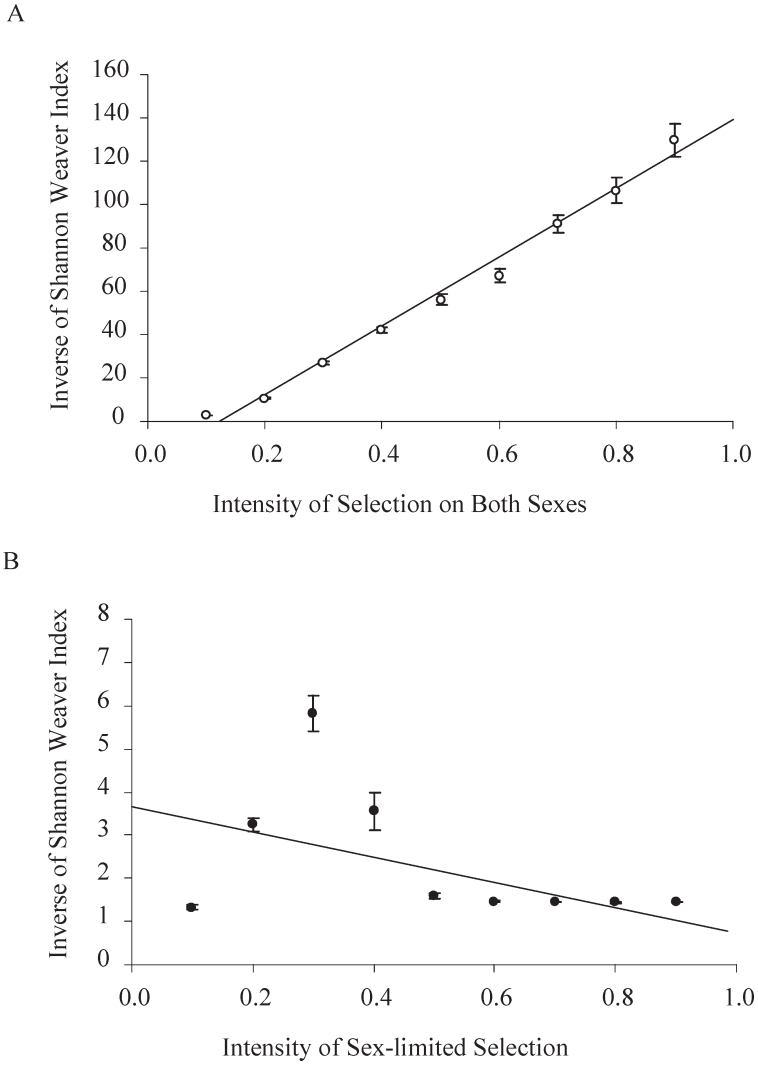

Fig. 5. To test the general notion that genetic variation varies inversely with the intensity of selection, we ran a regression on the reciprocal of the Shannon Weaver index versus selection intensity for (A) selection on both sexes and (B) sex-limited selection. Similar to genetic models, when selection influenced both sexes heritable variation correlated inversely with selection intensity. However heritable variation did not correlate inversely with selection intensity under the sex-limited selection scenario. Points represent the 1/variation maintained at equilibrium ($N = 100$ runs) for the array of selection intensities; error bars indicate the standard error.

increased with increasing selection intensity under the sex-limited temporally-fluctuating selection regime (Fig. 5B; Regression: $t = -8.4$, $F = 70.3$, $P < 0.001$, $R^2 = 0.269$, $df = 898$).

Because selection on both sexes influences the whole population, while sex-limited selection only influences half the population (the males), we tested and rejected the hypothesis that the variation maintained by sex-limited selection is equal to the variation maintained by selection on both sexes at half the selection intensity. We found significantly greater variation maintenance by sex-limited selection at all but the lowest selection intensity (Fig. 6; ANOVA: $R^2 = 0.871$, $df = 792$, Selection Intensity: $F = 24.6$, $P < 0.001$, Selection Type: $F = 1693.3$, $P < 0.001$, Type x Intensity Interaction: $F = 243.6$, $P < 0.001$).

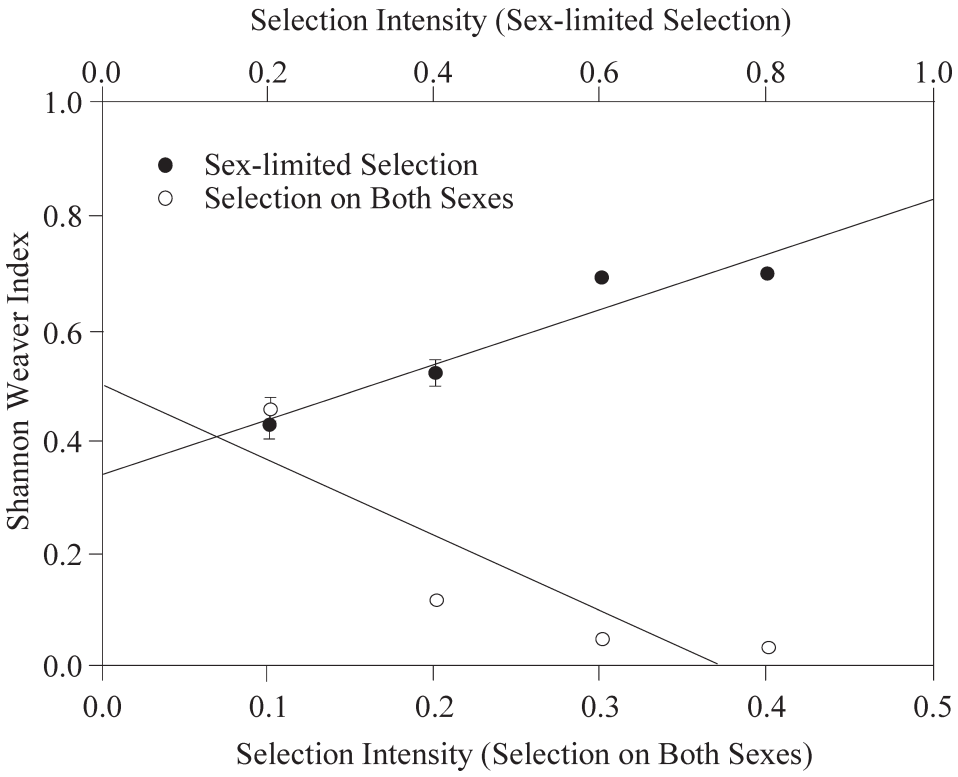

Fig. 6. To test the simple hypothesis that sex-limited selection is equivalent to selection on both sexes at half the selection intensity, we compared the results from selection influencing both sexes ($I = 0.1, 0.2, 0.3, 0.4$) with results from sex-limited selection ($I = 0.2, 0.4, 0.6, 0.8$). Sex-limited selection resulted in significantly greater variation being maintained in the population under all but the lowest selection intensity. Points represent the average variation maintained at equilibrium (N = 100 runs); error bars indicate the standard error.

Discussion

Many have argued that temporally fluctuating selection is incapable of maintaining genetic variation in a population, unless there is heterozygote advantage or generation overlap to provide a 'storage effect'. Following the expectations of Sasaki and Ellner (1997) and the genetic models of Reinhold (Reinhold 1999; 2000), we assert that even when generations are discrete, sex-limited temporally fluctuating selection can maintain substantial heritable variation. Elsewhere, one of us has also provided empirical evidence for sex-limited temporally fluctuation helping to maintain heritable variation in the Texas field cricket, which has discrete generations (Bertram 2002). Together these results strengthen the hypothesis that sex-limited temporally fluctuating selection can maintain heritable variation because they address the same question, using two very different modeling techniques and assuming two very different modes of inheritance.

Our phenotypic model produced results commensurate with previous models. Consistent with all previous research (Kimura 1954; Dempster 1955; Haldane and Jayaker 1963; Gillespie 1982; Gillespie 1989), we found that when selection influenced both sexes and fluctuated temporally, virtually no variation was maintained at equilibrium under all but a few very low-intensity selection regimes. However, following Reinhold (2000), we also found sex-limited selection to result in variation maintenance under all symmetrical selection regimes and under most asymmetrical cases. Note however, our definition of asymmetric fluctuating selection differs from that of Reinhold. In our model, the cumulative (or mean) selection over many generations is zero, i. e. no net directional selection. In Reinhold's (2000) model, however, asymmetric fluctuating selection means that selection intensity varies every other generation, but that the selection intensity values do not sum to one, i. e. there is fluctuating selection plus net directional selection.

When sex-limited selection is strong and changes direction frequently, substantial variation is maintained in the population. When selection changes direction less often, minimal variation is maintained by sex-limited selection because the average magnitude of selection is reduced. The way we have modeled asymmetric fluctuating selection was to keep a large negative selection intensity for one generation (we used –0.9 in our simulations) and then let the total accumulated variation over all other generations offset that initial selection. Thus, with the periodicity of fluctuating selection being ten generations, we had the last nine generations with selection intensities of positive one-tenth. Therefore, the average magnitude of selection intensity for those ten generations was one fifth the value we had for the case when selection intensity switched sign each generation. A similar result would hold if we computed harmonic (versus arithmetic) averages of the magnitude of selection intensity. Regardless, negligible

average selection tilts the balance in favor of mutation in any mutation-selection imbalance. Thus, both curves depicting heritable variation in Figure 4 (with and without sex-limited selection) begin increasing as the effects of mutation exceed those of selection.

What is new here is that we present a phenotypically based model for the maintenance of heritable variation via sex-limited temporally fluctuating selection. Our use of a phenotypic model allowed us to incorporate genetic, epigenetic, and non-molecularly based parent-of-origin effects. This is essential because heritabilities are computed using phenotypes, hence can reflect any combination of these effects (Gorelick and Bertram submitted). Traditional heritability measures do not and cannot discern between genetic and epigenetic inheritance (e. g. Giancotti et al. 1995; Van Speybroeck 2000; Finnegan 2002). Because our model can accommodate both genetic and epigenetic phenomena and shows maintenance of heritable variation, it provides strong support for the hypothesis that temporally fluctuating sex-limited selection can maintain heritable variation in a population.

Temporally fluctuating sex-limited selection is likely to be widespread; both environmental fluctuations and fluctuations in population density are ubiquitous and can lead to temporal fluctuations in the direction of selection (reviewed by Reinhold 1999; 2000). Because our model reveals that variation can be maintained in a population even when sex-limited selection changes direction after numerous generations of weak positive selection, we believe that temporally fluctuating selection accounts for some of the large heritable variance exhibited by sexual characters.

We assert that sex-limited selection can readily arise as a result of genomic imprinting because of the inherent differential methylation between females and males, methylation that can play an enormous role in regulation and development. No earlier models of sex-limited temporally fluctuating selection accounted for genomic imprinting or any other epigenetic effects. Therefore, it is entirely understandable that many of these earlier models predicted that no significant heritable variation could be maintained with sex-limited temporally fluctuating selection and discrete generations. Our phenotypic model subsumes both genetic and epigenetic effects. It therefore provides hints that *population genetic and epigenetic* models could account for this maintenance of heritable variation. This provides impetus for us and other researchers to develop such population epigenetic models.

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