Rapid evolution towards equal sex ratios in a system with heterogamety

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ABSTRACT

The equal sex ratios found in many species with heterogametic sex determination may be a consequence of selection for equality or the result of the Mendelian segregation of the two sex chromosomes. A lack of genetic variation in sex ratio in species with heterogamety has been the major obstacle in distinguishing between these two hypotheses. We overcome this obstacle by generating hybrids between two species of *Drosophila*. The resulting hybrid lines had biased sex ratios, allowing us to observe the evolution of sex ratio in replicate populations. Sex ratio converged towards 1:1 after 16 generations of natural selection. These changes in sex ratio were not due to differences in viability between the sexes and the loci underlying the variation in sex ratio were not sex-linked. Equal sex ratios may therefore be the result of natural selection as Fisher predicted.

Keywords: heterogamety, interspecific hybrids, natural selection, sex ratios.

INTRODUCTION

Two competing hypotheses are invoked to explain the ubiquity of 1:1 sex ratios (Bull and Charnov, 1988). Fisher (1930) suggested that frequency-dependent selection maintains equal sex ratios when the sexes cost the same to produce, resulting in a balance of investment between males and females. This adaptive hypothesis has received support in two studies on fish, one species with environmental sex determination (Conover and Van Vororhees, 1990) and the other with a three-factor sex determination system (Basolo, 1994). An alternative non-adaptive hypothesis in species with heterogamety is that Mendelian segregation of the sex chromosomes may account for 1:1 sex ratios (Maynard Smith, 1978; Toro and Charlesworth, 1982; Bull and Charnov, 1988). Selection for equal sex ratios has not been demonstrated in any species with heterogametic sex determination, with the exception of the accumulation of poylgenic autosomal suppressors of the segregation distorter locus (*SD*) in *Drosophila melanogaster* (Lyttle, 1979).

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The major obstacle to the study of sex ratio evolution in organisms with heterogametic sex determination has been the lack of genetic variation in sex ratio (Bull and Charnov, 1988; Basolo, 1994). Standard quantitative genetic techniques have been unable to detect significant levels of genetic variation in this trait (Toro and Charlesworth, 1982) until recently (Varandas *et al.*, 1997). The absence of genetic variation in sex ratios has been interpreted as evidence for the inability of sex ratios to evolve and, in turn, as support for the Mendelian segregation hypothesis (Toro and Charlesworth, 1982; Bull and Charnov, 1988). Although sex ratios could not evolve without genetic variation, the lack of evidence for genetic variation does not constitute direct evidence for or against either hypothesis. The absence of genetic variation in many systems may have been a consequence of strong selection for equal sex ratios (Bull and Charnov, 1988), and is therefore consistent with both Fisher's adaptive explanation and the non-adaptive alternative of Mendelian segregation.

One way to study the evolution of traits with little genetic variation is to hybridize closely related species, allow the genes to segregate into a number of iso-female lines, and then track their evolution by repeated measurements on replicate lines. Here, we report an experiment using hybrids between sympatric Australian *Drosophila serrata* and *Drosophila birchii*. Recombination and segregation generated genetic variation between replicate hybrid lines, resulting in lines with initially biased sex ratios. We demonstrate the rapid evolution of sex ratios towards equality in these hybrid lines.

METHODS

Drosophila serrata and *D. birchii* have very different, but overlapping, geographic distributions and habitat preferences, and are strongly sexually isolated (Dobzhansky and Mather, 1961; Ayala, 1965). However, when interspecific crosses are successful, offspring of both sexes are viable and fertile (Ayala, 1965). One successful mating between a *D. serrata* female × *D. birchii* male (S♀ × B♂) and one from the reciprocal cross (B♀ × S♂) were generated (see Blows, 1998, for details). From each female, 15 F_1 female progeny were collected as virgins and sib-mated to a single male. Each pair founded an iso-female line, 30 lines in total, which were maintained in one culture bottle each, at $N > 100$ for 35 generations at 25°C.

At the 8th, 24th and 35th generations after hybridization (denoted G8, G24 and G35, respectively), we measured the sex ratio and viability of the lines. Viability at G8 was determined by allowing generation 7 adults to lay for 20 h, then placing five eggs in each of nine vials (replicates) for each iso-female line, and recording the number surviving to pupation after 8 days. The vials were placed in three randomized complete blocks at 25°C. After eclosion, the sex ratio in the nine vials for each line was recorded as the proportion of males in the sample. The same procedure was used at G35, but with 9–11 replicate vials for each iso-female line. The viability and sex ratio at G24 were measured in a similar manner, with the exception that 20 eggs in each of 4–6 replicate vials for each iso-female line were used, and viability was scored at eclosion. The mid-parent viability was determined in the G8 experiment, with 21 replicates for each of *D. serrata* and *D. birchii*.

RESULTS AND DISCUSSION

The mean sex ratio and viability of the hybrid iso-female lines originating from each of the reciprocal interspecific crosses are presented in Table 1. A Mann-Whitney *U*-test on the line

Table 1. Means (± standard deviation) and sample sizes for sex ratio and viability of the hybrid iso-female lines originating from each of the reciprocal interspecific crosses[a]

Trait	S♀ × B♂		B♀ × S♂	
	Proportion males	n	Proportion males	n
Sex ratio G8	0.469 ± 0.174	14	0.399 ± 0.246	14
Sex ratio G24	0.487 ± 0.062	14	0.448 ± 0.081	15
Sex ratio G35	0.556 ± 0.128	11	0.440 ± 0.132	13
	Proportion survived	n	Proportion survived	n
Viability G8	0.453 ± 0.216	14	0.212 ± 0.152	15
Viability G24	0.556 ± 0.101	14	0.457 ± 0.098	15
Viability G35	0.552 ± 0.132	11	0.401 ± 0.160	13

[a] Lines which failed to produce sufficient eggs to establish test vials at each generation were excluded from the analysis.

means at G8 indicated no difference between the reciprocal crosses, suggesting no sex-linked effect for sex ratio ($U_{14,14} = 86.0$, $P = 0.603$). Lines from the S♀ × B♂ cross had significantly higher viability than the reciprocal cross B♀ × S♀ ($U_{14,15} = 38.0$, $P = 0.003$), suggesting a strong sex-linked effect on this trait. The grand mean of all lines for sex ratio did not change from G8 to G24 ($U_{28,29} = 402.0$, $P = 0.949$). The grand mean of viability increased significantly ($U_{29,29} = 204.5$, $P < 0.001$) from G8 to G24, and was indistinguishable from the mid-parent viability (0.511) in the latter generation (t-test: $t_{28} = -0.303$, $P = 0.764$).

We examined genetic variation in sex ratio and viability at G8 using a binomial general linear model with a logit linking function to test for significant heterogeneity among the lines. The among-line variance from this experimental design approximates the genetic variance, falling between the traditional narrow and broadsense definitions of genetic variance (Blows, 1998). Significant heterogeneity among lines was displayed in both sex ratio ($P = 0.026$) and viability ($P < 0.001$).

We analysed the evolutionary trajectories from G8 to G24 (Fig. 1a) using circular statistics (Zar, 1996). Evolutionary trajectories may be used to understand the directional component of character evolution. The trajectories in Fig. 1a appear to converge towards intermediate values for both traits. Given this distribution of trajectories, the space in Fig. 1a was divided into four quadrants by lines described by the grand means of the two traits at G24 (0.467 and 0.505 for sex ratio and viability, respectively), and the analysis of trajectories was conducted for each quadrant separately. The G24 rather than the G8 means were chosen to define the quadrants, as they are a better estimate of the position of any equilibrium point which may be the endpoint of selection.

Sex ratios converged significantly towards 1:1 from G8 to G24 (Fig. 1a). The mean angle of the trajectories in the lower left-hand quadrant was 28.0°, with a mean vector length of $r = 0.79$, and represented a significant mean direction (Rayleigh z-test: $n = 8$, $z = 4.99$, $P < 0.005$). The mean angle of the trajectories in the upper left-hand quadrant was −28.0°, with a mean vector length of $r = 0.93$, and represented a significant mean direction ($n = 13$, $z = 11.24$, $P < 0.001$). These two angles were significantly different (Watson-Williams test:
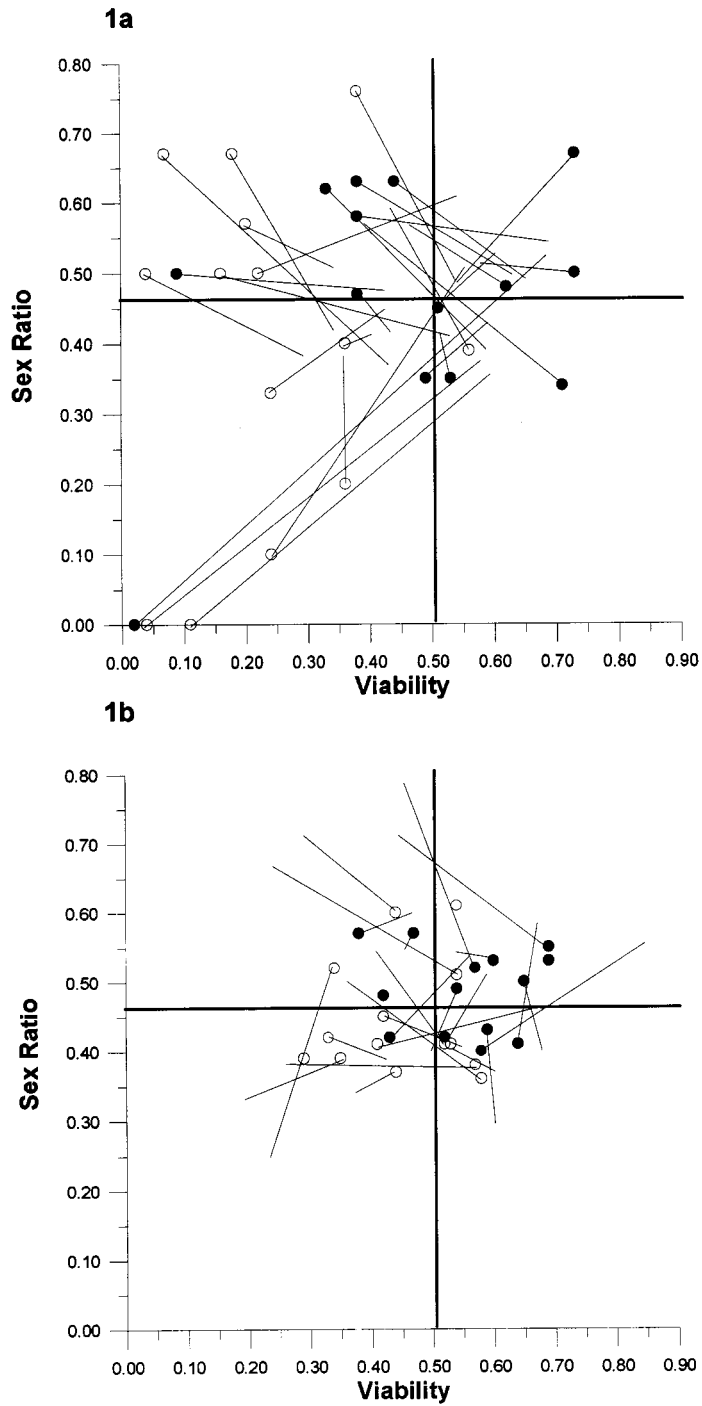
1a

1b

Fig. 1. Evolutionary trajectories of the hybrid iso-female lines: (a) from G8 to G24; (b) from G24 to G35. The initial position of each hybrid population is indicated by a closed circle (S♀ × B♂) or open circle (B♀ × S♂), and the final position is indicated by the end of the vector.

$F_{1,19} = 14.46$, $P < 0.005$), indicating that sex ratio was converging towards an intermediate value. Sex ratio in the two right-hand quadrants did not change significantly as a consequence of the small sample in each, but were consistent with convergence towards 1:1. The lower right-hand quadrant mean vector length was $r = 0.71$ (Rayleigh z-test: $n = 4$, $z = 2.02$, $0.20 > P > 0.10$), and the upper right-hand quadrant mean vector length was $r = 0.86$ ($n = 3$, $z = 2.22$, $0.20 > P > 0.10$).

Determining if a change in sex ratio towards 1:1 between G8 and G24 had occurred was also approached by using a paired t-test (pairing on the basis of line) on the absolute values of the differences between initial (G8) and final (G24) sex ratio from 0.5 for each line. The initial absolute differences were significantly larger than the final absolute differences ($t_{27} = 3.106$, $P = 0.004$, two-tailed), indicating convergence towards 1:1.

The between-line variance in sex ratio (F-test: $F_{27,28} = 8.32$, $P < 0.001$) and viability ($F_{28,28} = 4.00$, $P < 0.001$) were significantly reduced between G8 and G24. Since the G24 measurements were made on vials established with a larger number of eggs than those in G8, the reduction in between-line variance in sex ratio and viability may have been a consequence of sampling, rather than selection. Two approaches were used to test this contingency. First, the sex ratio and viability of the lines were again determined at G35 using the same number of eggs per vial as at G8 (Fig. 1b). There was no directional change from G24 to G35 in any of the four quadrants. This is consistent with the expectation that selection will be weak when sex ratios are near equality (Charnov, 1982; Bull and Charnov, 1988). The between-line variance in sex ratio at G35 was again significantly smaller than that found at G8 (F-test: $F_{27,23} = 2.29$, $P = 0.043$).

Second, we performed a bootstrap sampling procedure to determine if sampling alone could result in the decreased between-line variance in sex ratio (Fig. 2). The coefficient of variation (CV) of sex ratio was lower for G24 ($P < 0.001$) and G35 ($P = 0.006$) than for G8, and did not differ between G24 and G35 (mean difference ±95% confidence limits: 9.41 ± 12.56). Therefore, the reduction in variance in sex ratio from G8 to G24 cannot be attributed solely to the increase in sample size at G24. Similarly, sampling cannot account for the reduction in variance from G8 to G35, but sampling may be totally responsible for the apparent increase in variance from G24 to G35.

The CV of viability (Fig. 2) decreased from G8 to G24 ($P < 0.001$), but increased from G24 to G35 (mean difference ± 95% confidence limits: 11.20 ± 7.40). This implies that selection reduced variation in viability between G8 and G24, but some process increased variation from G24 to G35. This increase may have been due to the change in environment caused by the smaller number of eggs used at G35.

Finally, apparent evolutionary changes in sex ratio could occur simply as a consequence of changes in the viability of the sexes (Toro and Charlesworth, 1982). Haldane (1922) first noted that, in hybrid crosses, the heterogametic sex (males in these flies) was often absent, rare or sterile. If the observed evolution of sex ratio was simply due to an increase in male viability, we would expect a positive correlation between the line means for viability and the deviation of sex ratio from 1:1 at the start of the experiment. The correlation between the line means for viability and the deviation of sex ratio from 1:1 at G8 was not significant (Spearman's rank correlation: $r_s = -0.183$, $P = 0.351$, $n = 28$). Therefore, the biased sex ratios at G8 were not a consequence of differences in mortality between the sexes.

In conclusion, interspecific hybridization was used to generate genetic variation in sex ratio, and to perturb sex ratio away from equality in a series of hybrid lines. Natural selection rapidly returned the sex ratios of the hybrid lines towards 1:1, providing support
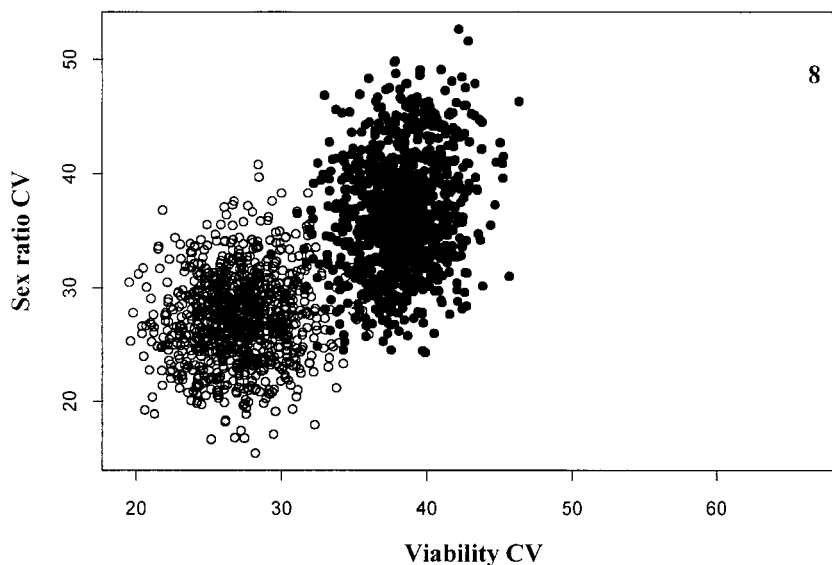
Fig. 2. Bootstrap analyses testing if the coefficient of variation (CV) of sex ratio and viability evolved in the hybrid lines. We sampled (with replacement) nine replicate vials of five eggs (as in G8) from the actual data from G24 (4–6 replicate vials containing 20 eggs) and G35 (9–11 replicate vials containing five eggs). This procedure was repeated 1000 times for each data set to generate an empirically derived distribution of variances for sex ratio and viability. Open circles are bootstrapped data from G24, closed circles are from G35, and the position of G8 is indicated by the numeral 8.

for Fisher's adaptive hypothesis for sex ratio evolution in organisms with heterogametic sex determination. No evidence for sex-linkage was found for sex ratio, suggesting that loci on autosomes may play an important role in sex ratio evolution.

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