

STABILIZING SELECTION AND VARIANCE IN FIG WASP SEX RATIOS

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Abstract.—Theory predicts that the phenotypic variance observed in a trait subject to stabilizing selection should be negatively correlated with the trait's impact on fitness. However, this relationship has rarely been tested directly. The offspring sex ratios produced by pollinating fig wasp foundresses upon entrance to a fruit and oviposition alone (single foundress sex ratios) are subject to stabilizing selection because too many males reduce the total number of dispersing females and too few males will result in unmated females or complete loss of the brood. Furthermore, we argue that the impact on fitness of, and therefore the intensity of stabilizing selection on, single foundress sex ratios are correlated to how frequently a species produces single foundress broods in nature. Specifically, the intensity of stabilizing selection will be greater in species that encounter single foundress broods more frequently, both because the trait is expressed more often and because fitness shows a greater sensitivity to variation (narrower fitness profile) when that trait is expressed. Across 16 species of Panamanian pollinating fig wasps, the phenotypic variance in single foundress sex ratios was negatively correlated with the frequency with which that species encounters single foundress broods in nature. In addition, a formal comparative analysis based upon a molecular phylogeny of the wasps gave results that were the same as when species were used as independent data points.

Key words.—Fig wasps, intensity of selection, sex ratio, stabilizing selection, variance.

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If a trait has an optimum value then, by definition, deviations from the optimum reduce fitness and so natural selection will be a stabilizing influence (Fisher 1930). However, traits show variation and the role that various factors can play in maintaining or reducing phenotypic variation has been the subject of much theoretical debate (reviewed by Bulmer 1985; Barton and Turelli 1989; Leigh 1990). Natural selection will reduce phenotypic variation in a trait subject to stabilizing selection either by reducing the genetic variation in the alleles coding for that trait (e.g., removing deleterious mutations) or by reducing the magnitude of the alleles' effects (termed canalization). In contrast, mutation, antagonistic pleiotropy (genes with multiple effects), fluctuating selection, migration and genotype-by-environment interactions (different genotypes have the highest fitness in different environments) can act to increase and maintain phenotypic variation. Nonetheless, the balance between these forces will depend ultimately upon the relative importance of the trait to fitness, suggesting that less phenotypic variance should be observed in traits that are more closely linked to fitness.

The relative importance to fitness of variation in a trait, and hence the intensity of stabilizing selection, is a product of two factors: the fitness profile of variation in that trait when it is expressed and the frequency with which that trait is expressed (Lande 1975; Turelli 1984). Traits whose effects on fitness show greater sensitivity to changes, as depicted by narrower fitness profiles, will be subject to greater stabilizing selection. The intensity of stabilizing selection should also be positively correlated to how frequently in nature the trait is expressed. In the extreme, there would be no selection on a trait that is never expressed, no matter how steep the fitness profile.

The predicted relationship between phenotypic variance and the intensity of stabilizing selection can be tested by two different, but complementary, methods. First, controlled laboratory experiments can be carried out on single species (e.g.,

Scharloo et al. 1967; Gibson and Bradley 1974; Stearns and Kawecki 1994; Stearns et al. 1995). For example, the phenotypic variance can be measured in a single trait whose impact on fitness is varied across experimental treatments or in a series of traits whose importance to fitness differs. Using this technique, Stearns and Kawecki (1994; Stearns et al. 1995) showed that the phenotypic variance observed in five fitness components of *Drosophila melanogaster* (age at eclosion, dry weight, early fecundity, late fecundity, life span) were correlated with their impact on fitness, and therefore the intensity of stabilizing selection. However, a second possible method is to adopt a comparative approach and to compare the natural phenotypic variation in a single trait across a series of species that have similar ecologies, but across which the intensity of stabilizing selection on the trait is likely to vary and can be quantified (see also Pomiankowski and Møller 1995).

In this paper, we adopt the second approach and compare the phenotypic variance in offspring sex ratios produced by females of different pollinating fig wasp species when they enter a fruit and oviposit alone (defined as single foundress sex ratio). When the offspring of only one or a few mothers mate among themselves in their natal patch, before daughters disperse, a female biased sex ratio is favored, by a process that has been termed local mate competition (LMC; Hamilton 1967). This female bias can be explained in terms of individual selection because it reduces the competition among sons for mates and because it increases the number of mates for each son (Taylor 1981). It can also be explained equally through hierarchical selection theory, which emphasizes the role of selection at different levels of the population structure (Hamilton 1979; Colwell 1981; Frank 1986). An additional bias is favored in haplodiploid species because inbreeding causes mothers to be more related to their daughters than their sons (Frank 1985; Herre 1985). Previous work has examined the mean sex ratios produced when different numbers

of female fig wasps enter a fruit, but not the variation between individuals (Herre 1985, 1987). When females oviposit alone, there is stabilizing selection on the offspring sex ratio (Green et al. 1982). We shall argue that the intensity of stabilizing selection varies across species in a quantifiable manner. Specifically, the intensity of stabilizing selection will be greater in species that encounter single foundress broods more frequently, both because the trait is expressed more often and because the fitness profile, when the trait is expressed, is narrower. In addition, we use a molecular phylogeny of the species being examined to carry out a formal comparative analysis and test for any phylogenetic effects on this relationship.

BACKGROUND BIOLOGY

Each species of fig is pollinated by a single species-specific pollinating wasp, the life cycles of which are very similar (Wiebes 1979, 1995; Herre et al. 1996). The fig species that we have examined here are in the subgenera *Urostigma* and *Pharmacosycea* and are pollinated by wasps in the genera *Pegoscapus* and *Tetrapus*, respectively. Individual trees of all these fig species may produce one to three fruit crops per year (Windsor et al. 1989). The development and maturation of the fruit within the crop of a single tree are synchronous. When the fruit are receptive, mated pollen-bearing female pollinating wasps (foundresses) arrive at the tree and enter these fruit. Importantly, different fig species have characteristically different distributions of foundress numbers per fruit (Herre 1985, 1987). The females then pollinate the receptive, uniovulate flowers and probe the flowers with their ovipositors attempting to lay eggs in the ovaries (Frank 1984; Herre 1989). Sex determination is haplodiploid, and thus females can control the sex of their offspring by whether or not they fertilize the egg (Cook 1993). These foundress wasps subsequently die inside the fruit, having produced only one brood. The bodies of the foundress wasps remain within the fruit and may be counted to determine the number of foundresses that pollinated and laid eggs in each fruit. Just before final ripening of the fruit, the wingless males of the pollinating wasps chew their way out of the flowers in which they have developed. They then crawl around the interior of the fruit searching for flowers that contain female wasps. The males chew open these flowers and mate with the females. The females emerge from their flowers and gather pollen, before leaving through a hole in the fruit wall chewed by the male wasps (Herre 1989, 1996). If there are not sufficient males in a brood to chew the exit hole then the females would not be able to leave their natal fruit, in which case the total brood fitness would be zero (Herre et al. 1997; West et al. 1997).

Stabilizing Selection for Precise Sex Allocation

The aim of this section is to demonstrate that there is stabilizing selection for precise sex allocation when only one foundress oviposits in a fruit (single foundress sex ratio), and that the intensity of stabilizing selection varies across species in a predictable manner. If only one female contributes offspring to a brood then the optimal offspring sex ratio (defined as proportion males) to produce is just enough sons to mate

the daughters (Hamilton 1967). In fig pollinating wasps, the males are also required to chew the exit hole in the fruit without which female offspring cannot leave their natal fruit (Herre et al. 1997). If we make the extreme assumption that one male is sufficient to inseminate all the females in a brood and chew an exit hole in the fruit, then the relative fitness return (w_1) in terms of mated female offspring from a brood of size b which contains m males, will be:

$$w_1 = (b - m)(1 - d)(1 - d^m), \quad (1)$$

where d is the probability of an individual dying before it reaches maturity (defined as the developmental mortality rate; Green et al. 1982). Here $(b - m)(1 - d)$ is the expected number of females reaching maturity, and $(1 - d^m)$ is the probability that at least one male survives. The optimal number of males to produce is found by solving $\partial w_1 / \partial m = 0$, which gives the equality

$$[(1 - d)(1 - d^m)] + [(1 - d)d^m(b - m)\log(d)] = 0, \quad (2)$$

which can be solved numerically for a given brood size and developmental mortality rate (for alternative approaches that predict integer numbers of males, see Nagelkerke and Hardy 1994; West et al. 1997). Example relationships of equations (1, 2) are shown in Figures 1 and 2, and illustrate two points: (1) the optimal number of males increases with both developmental mortality and brood size (Green et al. 1982; Nagelkerke and Hardy 1994; Nagelkerke 1996; West et al. 1997); and (2) there is stabilizing selection on the number of males (and therefore the sex ratio) to produce (Green et al. 1982). If too few males (sons) are produced, then the females (daughters) will remain unmated and an exit hole cannot be chewed in the fruit. If too many sons are produced, then resources are wasted that could have been used to produce extra females. If more than one male is required to mate all the females and chew an exit hole, then the same qualitative predictions are made, but with a greater number of males.

The strength of stabilizing selection can be quantified. In most areas of sex ratio evolution, selection is frequency dependent (Williams 1979). However, this is not the case for single foundress sex ratios, where all mating is between siblings, and so the fitness of a female is not affected by the sex ratio that other females produce. Consequently, the second differential of log fitness ($\partial^2 \log[w_1] / \partial m^2$) provides a measure of the strength of stabilizing selection (Lande and Arnold 1983; Turelli and Barton 1994), and equals

$$\partial^2 \log(w_1) / \partial m^2 = \frac{(d^m - 1)^2 + [d^m(b - m)^2(\log[d])^2]}{(d^m - 1)^2(b - m)^2}. \quad (3)$$

Note that more negative values imply stronger stabilizing selection. By numerically solving equation (2) and substituting into equation (3), it is possible to determine how the strength of stabilizing selection varies with brood size and the developmental mortality rate. Example relationships are given in Figure 3 and show that the strength of stabilizing selection decreases with increases in both brood size and developmental mortality. These results support and generalize those of Green et al. (1982), who compared the theoretical fitness of females who produce precise sex ratios with those who imprecisely produce sex ratios with a binomial

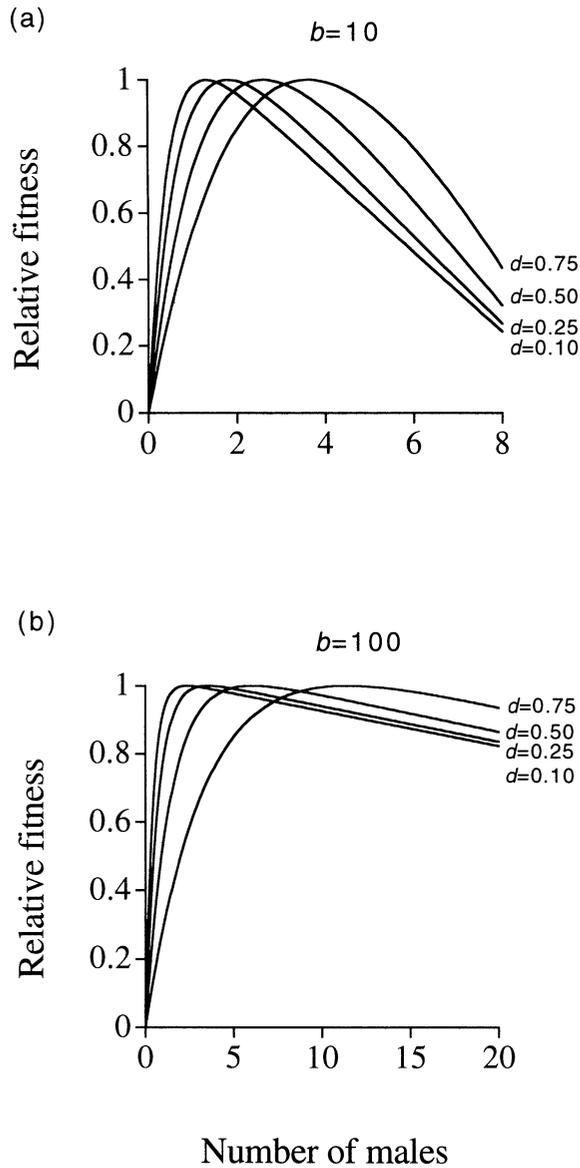


FIG. 1. The theoretical relationship (fitness profile) between fitness and number of males laid in a brood (m) when one female oviposits in a patch. Relationships are shown for various values of developmental mortality (d), and brood size (b): (A) $b = 10$; (B) $b = 100$. Relative fitness is plotted, with the fitness of the optimal strategy for each mortality rate (d) defined as 1.0.

variance. They showed that the production of precise sex ratios is advantageous because it leads to a higher number of inseminated females from broods of a given size, and that the advantage of precise sex ratios decreases as both brood size and developmental mortality increase.

The intensity of stabilizing selection on single foundress sex ratios is expected to be inversely related to how frequently females of a species oviposit alone. The distribution of numbers of foundresses per fruit varies across fig species (Table 1; Herre 1985, 1987). For example, while in some species almost all (> 0.99) of the broods are founded by only a single foundress, in other species the proportion of single foundress broods may be as low as 0.07. This variation has

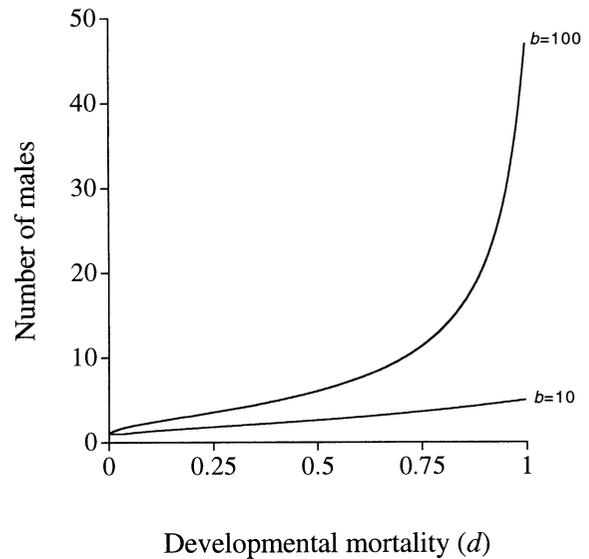


FIG. 2. The optimal number of males to produce when one female oviposits in a patch. The two lines represent different brood sizes (b).

two consequences that are important for the purposes of this paper. First, the more often single foundress broods occur, the more important the sex ratio produced in single foundress broods will be for the average female fitness. We would therefore expect greater stabilizing selection (Lande 1975; Turelli 1984). In the extreme case of a species in which single foundress broods never occurred there would be no selection on the single foundress sex ratio and any amount of variance in the single foundress sex ratio would be tolerated.

A second reason why a lower proportion of single foundress broods may reduce the intensity of stabilizing selection arises if females adjust their sex ratio according to the possibility that another female will also enter the fruit. In this case we shall show that the fitness profile is widened. The fitness return (w_2), in terms of mated daughters and number of mates obtained by sons, from a brood when there is a probability p that a second female will later enter the fruit and also lay a brood of size b , containing m_2 males, will be

$$w_2 = [2(1 - p)(b - m)(1 - d)(1 - d^m) + [p(1 - d)([b - m] + [2b - m - m_2]m/[m + m_2]) \times (1 - d^{m+m_2})]. \quad (4)$$

The first term represents the probability that no other female will enter the fruit multiplied by the fitness of a female ovipositing alone (eq. [1] multiplied by two, because we must now also include mates obtained by sons). The second term represents the probability that a second female enters the fruit multiplied by the fitness of the first female given that event. In this case, the fitness of the first female will be the number of daughters that she produces plus the number of mates obtained by her sons, multiplied by the probability that at least one male survives. As before, the optimal strategy of the first female is found by solving $\partial w_2/\partial m = 0$ and is given in the Appendix (eq. [A1]).

Sample relationships are shown in Figures 4 and 5 and

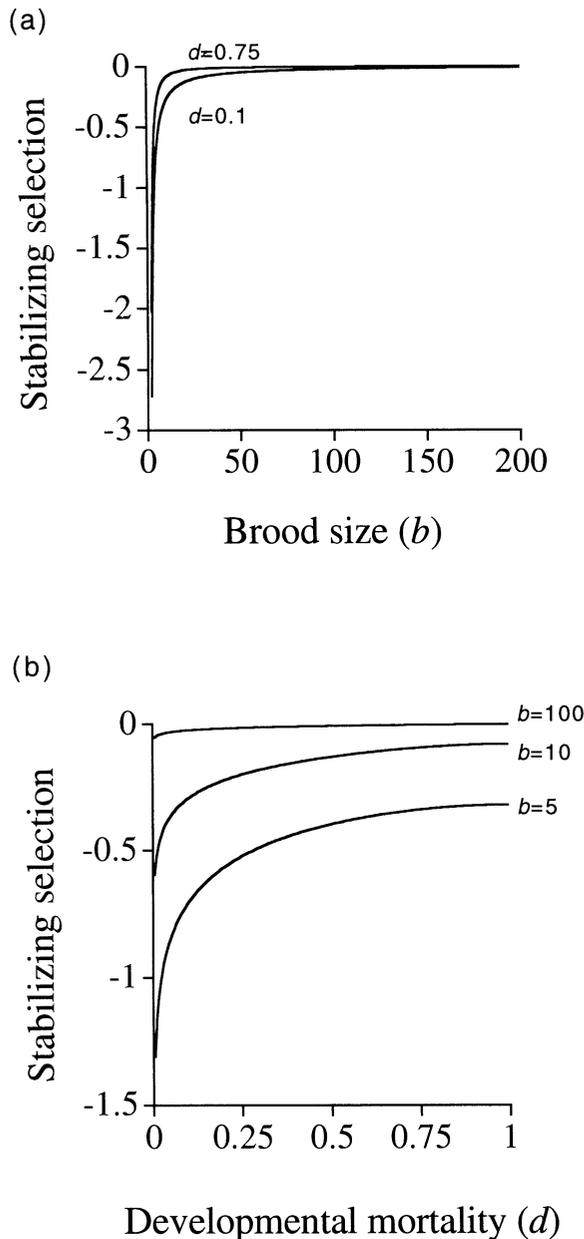


FIG. 3. The strength of stabilizing selection on the number of males to produce when only one female oviposits in a patch. (A) The consequences of variable brood size (b) for two values of developmental mortality (d). (B) The consequences of variable developmental mortality (d) for three values of brood size (b).

illustrate two points: (1) females ovipositing in a fruit are expected to lay a higher offspring sex ratio with an increasing probability of another female entering a patch (Werren 1980; Greeff and Compton 1996; Herre et al. 1997); and (2) an increasing probability of another female entering the patch leads to a lower chance of no males developing in a fruit, which widens the fitness profile and so decreases the intensity of stabilizing selection for precise sex ratios. As before, we can show this point quantitatively by using the fact that the second differential of log fitness ($\partial^2 \log[w_2]/dm^2$) provides a measure of the strength of stabilizing selection, and is given

in the appendix (eq. [A2]). Example relationships are given in Figure 6. The strength of stabilizing selection decreases with increasing brood size, developmental mortality, and the probability that a second female enters the fruit. These points hold even if the second female lays a smaller brood size or does not lay a less female biased sex ratio. Furthermore, there is a possibility that more than one other female will enter the fruit. Given this possibility, we would expect an even less male biased sex ratio to be favored and an even greater reduction in the intensity of stabilizing selection.

The importance of these effects will be determined by the relative occurrence of sequential and simultaneous oviposition by multiple foundresses. If all multiple foundress broods result from simultaneous oviposition, then females will not be selected to adjust their sex ratio according to the possibility of another female entering the fruit. Instead they would adjust their sex ratio according to the number of foundresses in the fruit, which is a separate issue (Frank 1985; Herre 1985). However, it is likely that some broods may result from sequential oviposition (Greeff and Compton 1996), and indeed we (Herre et al. 1997) have provided empirical support for the prediction that females ovipositing in a fruit are expected to lay a higher offspring sex ratio with an increasing probability of another female entering a patch.

To summarize, the intensity of stabilizing selection on single foundress sex ratios is expected to be greater in species that experience single foundress broods more often. Here we test this prediction by examining whether the variance in single foundress sex ratios correlates with the frequency of single foundress broods across 16 species of pollinating fig wasps. We also examine whether the variance in single foundress sex ratios shows any correlation with average sex ratio, average brood size, average number of males in a brood, or number of broods sampled to obtain the estimate of variance.

METHODS AND ANALYSES

Data Collection

We have examined the pollinating fig wasp species found near Barro Colorado Island, Panama. The data used in this study were collected as part of a long-term study investigating sex allocation in these species between January 1981 and February 1997 (Herre 1985, 1987, 1989; Herre et al. 1997). The distributions of numbers of foundresses per fruit for each species were determined by collecting 20 or more recently pollinated fruit from the fruit crops of several trees. These fruit were then dissected and the number of female foundresses counted. To determine the number of male and female progeny produced in fruit entered by only a single foundress, we collected samples of fruit late in the ripening cycle of the fruit crop before any wasps had left the fruit. Each fruit was then cut open and the number of foundresses counted. Fruit that contained single foundresses were sealed between two petri dishes, and all the wasps were allowed to emerge before being frozen. Later, the number and sex of each species of wasp that the fruit had contained were recorded. Broods that contained an exceptionally high proportion of males (0.5–1) were assumed to have been produced by sperm-depleted or virgin females (Godfray 1988, 1990; West et al. 1997, 1998), and so were not included in the analysis presented here. How-

TABLE 1. Characteristics of the single foundress fig wasp broods sampled in nature. GV and HF represent our two estimates of the degree of variance in the sex ratio. Values of GV or HF < 1 represent less than binomial variance in the sex ratio data, while values > 1 represent greater than binomial variance. The significance level of differences in GV from binomial variance are given by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fig species (<i>Ficus</i>)	Wasp species (<i>Pegoscapus</i> or <i>Tetrapus</i>)	Number of fruit crops sampled	Proportion fruits with single foundress broods	Number broods sampled	Mean brood size	Mean sex ratio	GV	HF
<i>columbrinae</i>	<i>P. orozcoi</i>	21	0.99	43	84	0.08	0.50**	0.54
<i>perforata</i>	<i>P. standleyi</i>	22	0.99	130	86	0.09	0.80***	0.85
<i>paraensis</i>	<i>P. herrei</i>	39	0.92	143	213	0.05	1.06	0.80
<i>pertusa</i>	<i>P. silvestri</i>	16	0.90	56	116	0.09	0.80	0.73
<i>obtusifolia</i>	<i>P. hoffmeyer</i>	48	0.83	69	397	0.05	1.04	1.09
<i>bullenei</i>	<i>P. sp.</i>	24	0.82	141	136	0.06	0.89	0.83
<i>citrifolia</i>	<i>P. tonduzi</i>	65	0.78	254	158	0.05	0.84*	0.85
<i>turbinata</i>	<i>P. sp.</i>	3	0.75	43	114	0.07	0.81	0.87
<i>maxima</i>	<i>T. sp.</i>	10	0.70	25	187	0.10	1.04	1.09
<i>yoponensis</i>	<i>T. ecuadoranus</i>	15	0.58	45	112	0.12	1.60**	1.59
<i>dugandi</i>	<i>P. longiceps</i>	15	0.56	55	127	0.07	0.98	1.11
<i>nymphaefolia</i>	<i>P. picipes</i>	33	0.55	112	242	0.05	1.46	1.57
<i>costaricana</i>	<i>P. sp.</i>	2	0.40	18	114	0.09	1.60	1.49
<i>nr. trigonata</i>	<i>P. lopesi</i>	36	0.31	45	279	0.08	2.18***	2.22
<i>insipida</i>	<i>T. sp.</i>	33	0.27	79	122	0.14	2.03***	2.38
<i>popenoii</i>	<i>P. gemellus</i>	61	0.24	80	143	0.10	1.24	1.28

ever, it should also be noted that such broods were rare (0–5 for each species) and that removal of these data did not change the overall qualitative form of the results.

Measuring Variance

We measured the variance in the sex ratio data using two methods. The rationale behind both methods was to compare the variance observed in the sex ratio data of a species with the expected variance given a binomial (random) distribution. The ratio of these two numbers (observed variance/expected variance) shows to what extent the data for that species has less (ratio < 1) or more (ratio > 1) than binomial variance.

First we used the regression method of Green et al. (1982). Green's method compares the variance (or in this case its estimate, the mean squared error [MSE]) of the number of males observed with that expected if the number of males were binomially distributed. The observed value of MSE is given by the equation

$$s^2(1 - r^2), \quad (5)$$

where s^2 is the variance of the number of males per brood, and r is the regression coefficient of the relationship between the number of males in a brood and total brood size (Green et al. 1982). The expected value is the value of the binomial variance, npq , averaged over the value of n , the brood sizes observed. The values of p (expected proportion males) and $q(1 - p)$ are given by the regression equation for number of males against brood size. The expected value is therefore given by the equation

$$\sum (npqz) / \sum z, \quad (6)$$

where z is the number of broods with n individuals (Green et al. 1982). We shall define this ratio of observed variance/expected variance to be the Green variance (GV).

A test may be performed on GV to determine whether it is significantly lower (precise sex allocation) or greater (ov-

erdispersed sex allocation) than expected if sex allocation were binomial. Under the null hypothesis of binomial sex allocation, the residual test statistic: $\chi^2 = GV(N - 2)$, should come approximately from a chi-squared distribution with $N - 2$ degrees of freedom, where N is the total number of broods examined in that species (Green et al. 1982).

Our second method of estimating variance used maximum likelihood techniques implemented in the GLIM statistical package (Crawley 1993). We assumed binomial error variance and a logit link function, with the number of males in a brood as the response variable, and the total number of males and females in a brood as the binomial denominator. We then used general linear modeling techniques (McCullagh and Nelder 1989) to fit the minimum adequate model (MAM) to the data. As explanatory variables we employed: (1) brood size and a quadratic ([brood size]²); and (2) the reciprocal of brood size (see Griffiths and Godfray 1988). After fitting the MAM to the data, the ratio of the residual deviance to the residual degrees of freedom (termed the heterogeneity factor [HF]) provides an estimate of how the observed variance compares with the expected, assuming the binomial distribution. Similar to GV, values of HF < 1 indicate less than binomial variance, and values of HF > 1 show more than binomial variance.

Comparison across Species

We analyzed the data by two methods: we used species as independent data points and we used the method of independent contrasts to carry out a formal comparative analysis (Burt 1989; Harvey and Pagel 1991). For each of these methods we carried out separate analyses with GV and HF as the dependent (Y) variable. GV and HF are not statistically independent and we did analyses with both to demonstrate that the conclusion was not dependent on the measure of variance used.

We carried out the species as independent data points anal-

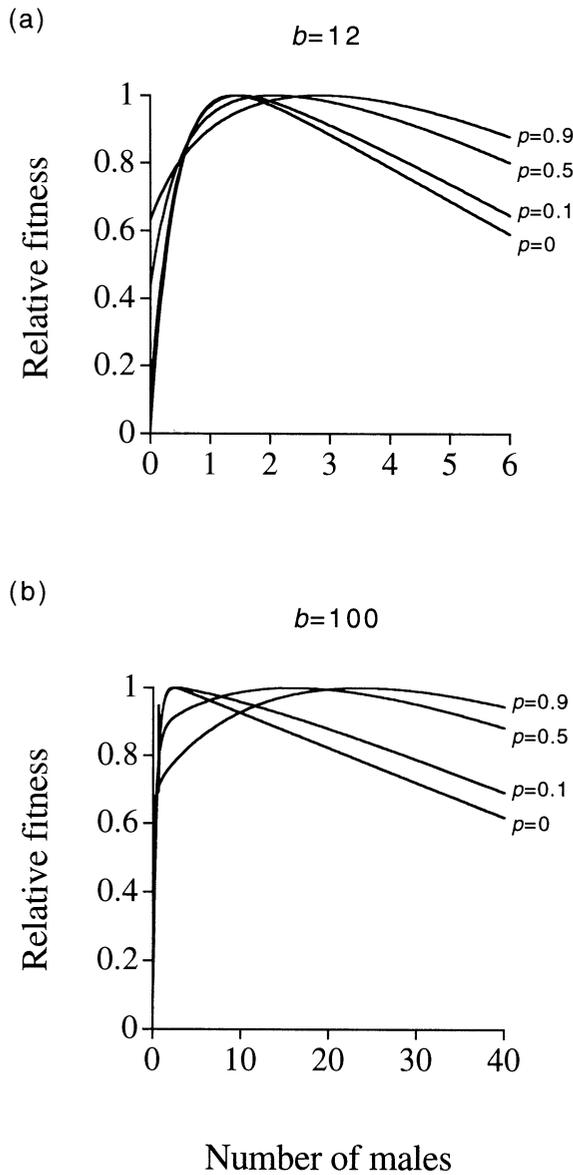


FIG. 4. The theoretical relationship (fitness profile) between fitness and number of males laid in a brood (m) when a female oviposits in a patch with a probability p that a second female will also oviposit in the same patch. In both graphs the developmental mortality (d) is assumed to be 0.1. The brood sizes (b) of both females and the number of males laid by the second female (m_2) for the two graphs are: (A) $b = 12$, $m_2 = 3$; (B) $b = 100$, $m_2 = 25$. As in Figure 1, relative fitness is plotted, with the fitness of the optimal strategy, for each probability p , defined as 1.0.

ysis using multiple regression techniques available in the GLIM statistical package. The average brood size, average number of males in a fruit, arcsine square root of average sex ratio, arcsine square root of the proportion single foundress broods, and the number of broods sampled to obtain the estimates of variance were used as explanatory variables. Nonlinearity in any relationships were tested for by the addition of quadratic terms.

Species may not be independent data points because they are phylogenetically related (Felsenstein 1985). This leads to problems if a trait is evolutionarily conserved or highly cor-

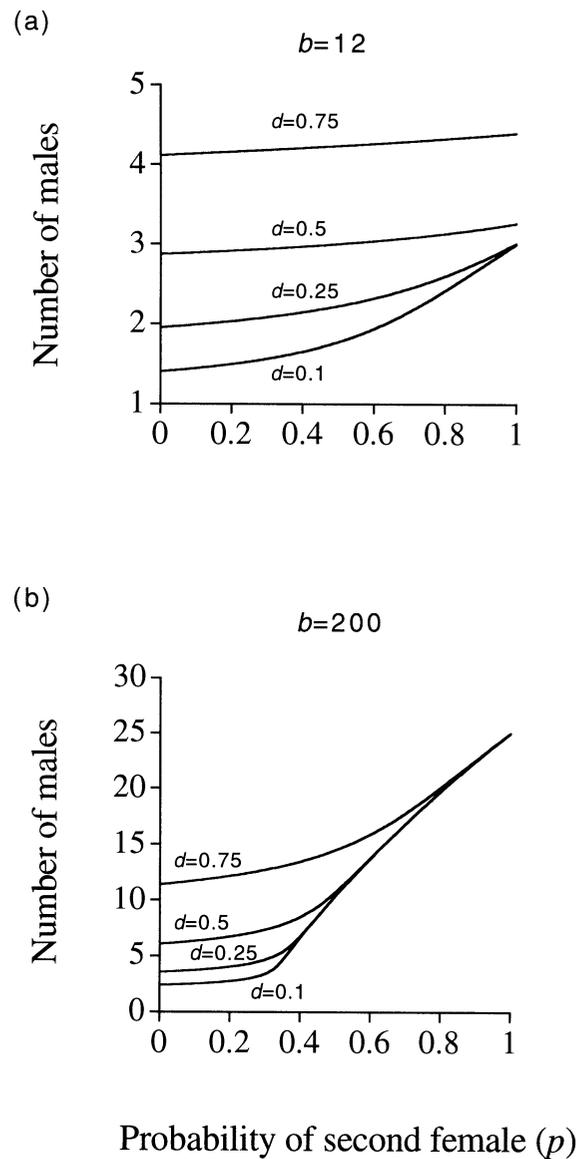
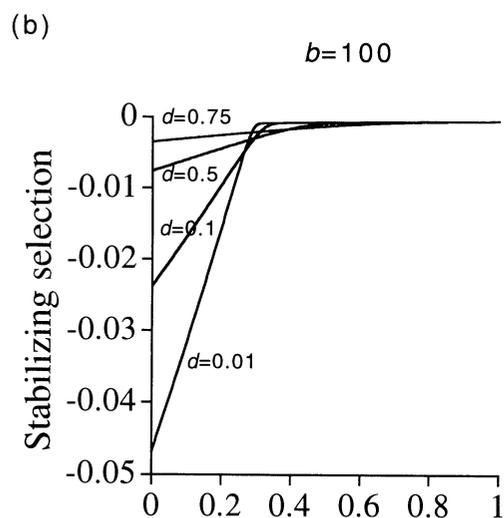
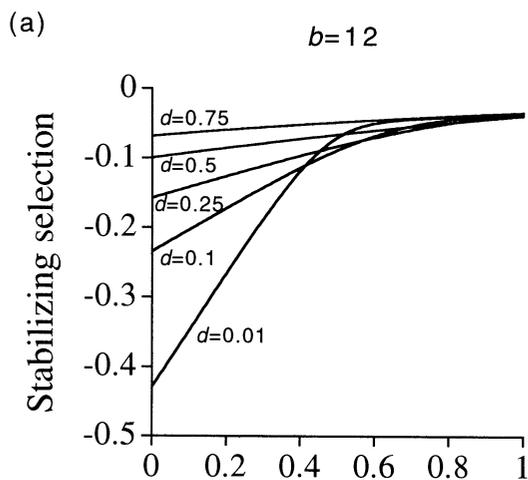


FIG. 5. The optimal number of males to produce when a female oviposits in a patch with a probability p that a second female will also oviposit in the same patch. Different lines represent different rates of developmental mortality. The brood sizes of both females (b) and the number of males laid by the second female (m_2) for the two graphs are: (A) $b = 12$, $m_2 = 3$; (B) $b = 100$, $m_2 = 25$.

related with an unknown phylogenetically inert third variable. Put simply, closely related species tend to share many characters through common descent rather than through independent evolution. The most popular modern comparative methods attempt to circumvent this problem by using the phylogeny of the species being examined to construct a series of independent contrasts. Independent contrasts are derived by calculating the difference in the response and explanatory variables across pairs of species or higher nodes that share a common ancestor (Felsenstein 1985; Grafen 1989; Pagel 1992). Hypothesis testing can then be carried out on these contrasts with a regression fixed through the origin or a sign test (Harvey and Pagel 1991; Garland et al. 1992). The ex-



Probability of second female (p)

FIG. 6. The strength of stabilizing selection on the number of males to produce when a female oviposits in a patch with a probability p that a second female will also oviposit in the same patch. Different lines represent different rates of developmental mortality. The brood sizes (b) of both females and the number of males laid by the second female (m_2) for the two graphs are: (A) $b = 12$, $m_2 = 3$; (B) $b = 100$, $m_2 = 25$.

pected value of the slope through the origin equals the true relationship between the variables in the absence of phylogenetic effects (Pagel 1993). We carried out a formal comparative analysis based upon a molecular phylogeny of the Panamanian fig pollinating wasps constructed by Machado et al. (unpubl. manuscript; Herre et al. 1996), and using the CAIC statistical package (Purvis and Rambaut 1995). The phylogeny was constructed using 1600 base pairs from the COI and COII mitochondrial genes. All variables were transformed before analysis, as when using species as independent data points.

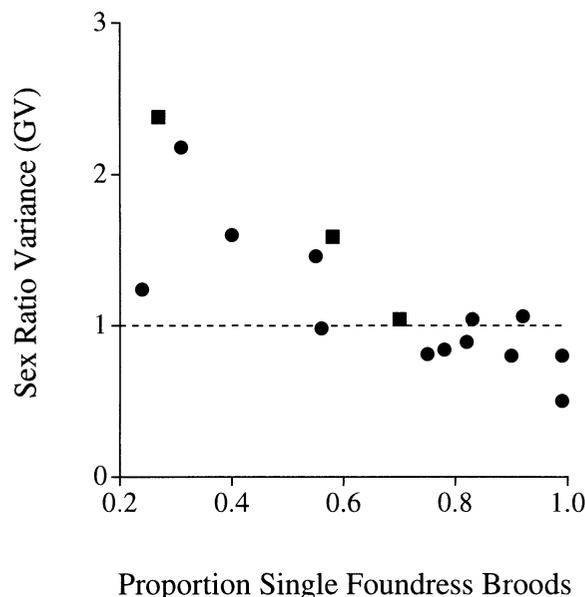


FIG. 7. The relationship between variance in single foundress sex ratios (GV) and the proportion of single foundress broods encountered in nature by that species. The circular points represent species in the genus *Pegoscapus* and the square points represent species in the genus *Tetrapus*. The dashed line represents binomial variation in the sex ratio (GV = 1).

RESULTS

The characteristics of the single foundress fig wasp broods sampled for the 16 species are given in Table 1. As can be seen, in all species the mean brood sizes were relatively large (84–397), and the mean sex ratios extremely female biased (0.05–0.14). Only three broods contained no males: two of *Pegoscapus hoffmeyer* and one of *P. tonduzi*. The estimates of variance in the sex ratio ranged from significantly less than binomial variance to significantly greater than binomial variance.

Species as Data Points

The variance estimates obtained by the two methods were highly positively correlated ($F_{1,13} = 167.17$, $P < 0.01$, $r^2 = 0.93$, $n = 16$; Table 1) and gave qualitatively identical results when used as dependent variables. The variance observed in the single foundress sex ratios of a species were negatively correlated with the frequency with which that species encounters single foundress broods in nature (GV: $F_{1,14} = 21.44$, $P < 0.01$, $r^2 = 0.60$; HF: $F_{1,14} = 22.71$, $P < 0.01$, $r^2 = 0.62$; Fig. 7, Table 2), and showed no significant relationship with brood size (GV: $F_{1,13} = 0.62$, $P > 0.05$; HF: $F_{1,13} = 0.32$, $P > 0.05$), number of males in a brood (GV: $F_{1,13} = 2.81$, $P > 0.05$; HF: $F_{1,13} = 2.39$, $P > 0.05$), sex ratio (GV: $F_{1,13} = 1.29$, $P > 0.05$; HF: $F_{1,13} = 1.57$, $P > 0.05$), or the number of broods sampled (GV: $F_{1,13} = 0.10$, $P > 0.05$; HF: $F_{1,13} = 0.13$, $P > 0.05$).

Formal Comparative Analysis

All following analyses were carried out on the calculated independent comparisons and, unless otherwise stated, rep-

TABLE 2. Slopes of relationships between the estimates of single foundress sex ratio variance (GV or HF) and the (arcsine square-root transformed) proportion of single foundress broods. Results are given for analysis using species as independent data points (Species) and the method of independent contrasts (IC).

Analysis type	Y variable	Proportion single foundress broods	
		Slope	SE
Species	GV	-0.85	0.18
IC	GV	-0.77	0.24
Species	HF	-0.96	0.20
IC	HF	-0.99	0.25

resent regressions fixed through the origin. The variance estimates obtained by the two methods were highly positively correlated ($F_{1,14} = 11.15$, $P < 0.01$, $r^2 = 0.90$, $n = 16$; Table 1) and gave qualitatively identical results when used as dependent variables. The variance observed in the single foundress sex ratios of a species were negatively correlated with the frequency with which that species encounters single foundress broods in nature (GV: $F_{1,14} = 13.98$, $P < 0.01$, $r^2 = 0.50$; HF: $F_{1,14} = 15.12$, $P < 0.01$, $r^2 = 0.52$; Fig. 8; Table 2) and showed no significant relationship with brood size (GV: $F_{1,13} = 2.41$, $P > 0.05$; HF: $F_{1,13} = 1.81$, $P > 0.05$), number of males in a brood (GV: $F_{1,13} = 3.63$, $P > 0.05$; HF: $F_{1,13} = 4.38$, $P > 0.05$), sex ratio (GV: $F_{1,13} = 0.01$, $P > 0.05$; HF: $F_{1,13} = 0.14$, $P > 0.05$), or the number of broods sampled (GV: $F_{1,13} = 0.07$, $P > 0.05$; HF: $F_{1,13} < 0.01$, $P > 0.05$). Significant negative correlations between the variance in single foundress sex ratios and the proportion of single foundress broods in nature were also found when analysis was carried out with sign tests on the independent contrasts (GV: $P < 0.01$; HF: $P < 0.01$; Fig. 4).

DISCUSSION

Across species, the variance in single foundress sex ratios (measured as the ratio of observed variance/expected variance given a binomial distribution) was negatively correlated with the frequency with which that species encounters single foundress broods in nature (Figs. 7, 8). In contrast, the variance in single foundress sex ratios showed no significant correlation with any other variable: the average sex ratio, average brood size, average number of males in a brood, and number of broods sampled to obtain the estimate of variance. We presented a model that suggested that the intensity of stabilizing selection on single foundress sex ratios increases with the frequency of single foundress broods. This happens both because the trait must be expressed more often and because the fitness profile, when that trait is expressed, narrows (Fig. 6). Given this argument, our result supports the prediction that the phenotypic variation observed in a trait will be negatively correlated to the intensity of stabilizing selection on that trait. Further, all analyses using species as independent data points gave slopes that were not significantly different from the analyses with independent contrasts (Table 2), implying that there were no, or small, phylogenetic effects (Pagel 1993).

The advantage of precise sex allocation increases with decreasing brood size, when less males are favored (eqs. [3,

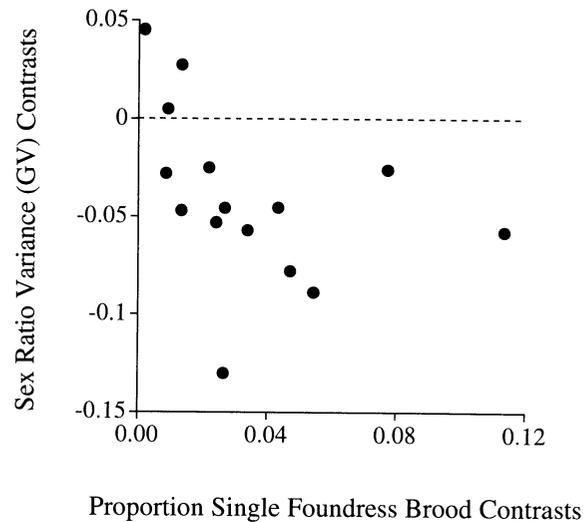


FIG. 8. The relationship between independent contrasts of variance in single foundress sex ratios (GV) and the contrasts of (arcsine square-root transformed) proportion of single foundress broods encountered in nature.

A2]; Figs. 3, 6; Green et al. 1982). In contrast to this prediction, we found no significant correlation between the variance in single foundress sex ratios and either average brood size or number of males in a brood. However, it should be noted that the correlation between variance in single foundress sex ratios and number of males in a brood was positive, as predicted, and close to significance in all analyses ($P < 0.1$). A possible explanation for the lack, or weakness, of this relationship is that the relative advantage of precise sex allocation varies little over the range of brood sizes found in the species considered here. These species have relatively large brood sizes (> 80 ; Table 1), and our results (Fig. 3) suggest that variation in brood size becomes less important with large brood size, particularly those greater than 50.

While some of the species that we have examined show precise sex allocation (significantly less than binomial variance), others have overdispersed sex allocation (significantly greater than binomial variance; Table 1). Precise sex allocation is theoretically advantageous when producing single sex broods. Our results are consistent with several laboratory studies demonstrating precise sex allocation in parasitoid and mite species in which single foundress oviposition is likely to be common (Green et al. 1982; Hardy 1992; Morgan and Cook 1994; Hardy and Cook 1995; Nagelkerke 1996). In contrast, overdispersed single foundress sex ratios are not predicted by theory, and so their observation are perhaps surprising (see Pickering [1980] and Rosenheim [1993] for when overdispersed sex ratios might be expected in outbred populations and West et al. [unpubl. data] for the consequences). One possibility, which we discuss below, is that selection may fluctuate in space and time. Another possibility is if females cannot judge their environment perfectly (incomplete knowledge) then greater variance may be expected (Antolin et al. 1994; Flanagan et al., in press). However, a crucial point is that we have examined secondary sex ratios (i.e., after developmental mortality) and not the primary sex ratio females attempted to produce. Processes such as sperm

depletion in females (Godfray 1990; West et al. 1998) or developmental mortality caused by a lack of resources in the fruit and competition with nonpollinating fig wasps (Herre 1989, 1996; West and Herre 1994; West et al. 1996), could have occurred in all species, adding extra noise to the secondary sex ratio data. Consequently, we are likely to have underestimated the degree of precision in single foundress sex ratios.

To what extent is the phenotypic variation in single foundress sex ratios likely to reflect genetic or environmentally induced variation? Genetic variation for single foundress sex ratios has previously been found within populations of the parasitoid wasp *Nasonia vitripennis* (Orzack et al. 1991). The phenotypic variation observed here would reflect genetic variation if all females of a species produced their broods under identical conditions. The closed environment of the fig fruit, and oviposition alone, would have provided as constant an environment as is possible under field conditions. However, developmental conditions may vary between trees, and the foundresses pollinating a single fruit crop would have developed in the fruit of several different trees (Nason et al. 1996). In addition, as mentioned above, we have measured secondary sex ratios. Consequently, the phenotypic variation we have observed is likely to reflect both genetic and environmentally induced variation.

What could maintain genetic variation in single foundress sex ratios? Theoretical studies suggest that additive genetic variance in traits subject to stabilizing selection can be maintained by several mechanisms including mutation-selection balance, fluctuating selection in space and time, migration, pleiotropy and genotype-by-environment interactions (Barton and Turelli 1989; Kondrashov and Yampolsky 1996; Phillips 1996; Lythgoe 1997). Although we have no idea of the relevance of the other mechanisms, we do know that selection is likely to fluctuate spatially and over time for the purpose of this study. Flower development rates and the frequency of competing or parasitic nonpollinator wasps vary between trees and even between fruit on the same tree (Herre 1989, 1996; West and Herre 1994; West et al. 1996; Herre and West, in press). This will lead to variable mortality rates, that will in turn favor different sex allocation strategies (eqs. [1–4, A1]; Green et al. 1982; Nagelkerke and Hardy 1994).

Herre (1987) has already shown the importance of selective regime (distribution of number of foundress mothers per fruit) in shaping the mean sex ratios of the species studied here under variable conditions of LMC (variable numbers of foundresses entering a fruit). Specifically, he showed that the mean sex ratios of the species were closest to theoretical predictions in the situations that they encounter most frequently, and that the ability of females to alter their brood sex ratios in response to variable LMC is most pronounced in species that frequently encounter variable LMC. In this paper we have shown that the selective regime also plays a role in shaping sex ratio variance. Together, these results demonstrate the limits of natural selection in creating perfect organisms and emphasize the importance of understanding the contemporary selective regime when designing and interpreting experimental work (Herre 1987; Kondrashov and Houle 1994; Pomiankowski and Møller 1995; Herre et al. 1997).

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LITERATURE CITED

- ANTOLIN, M. F., P. J. ODE, AND M. R. STRAND. 1994. Variable sex ratios and ovidice in an outbreeding parasitic wasp. *Anim. Behav.* 49:589–600.
- BARTON, N. H., AND M. TURELLI. 1989. Evolutionary quantitative genetics: how little do we know? *Annu. Rev. Genet.* 23:337–370.
- BULMER, M. G. 1985. *The mathematical theory of quantitative genetics*. Oxford Univ. Press, Oxford.
- BURT, A. 1989. Comparative methods using phylogenetically independent contrasts. *Oxf. Surv. Evol. Biol.* 6:33–53.
- COLWELL, R. K. 1981. Group selection is implicated in the evolution of female-biased sex ratios. *Nature* 290:401–404.
- COOK, J. M. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* 71:421–435.
- CRAWLEY, M. J. 1993. *GLIM for ecologists*. Blackwell Scientific Publications, Oxford.
- FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125:1–15.
- FISHER, R. A. 1930. *The genetical theory of natural selection*. Oxford Univ. Press, Oxford.
- FLANAGAN, K. E., S. A. WEST, AND H. C. J. GODFRAY. In press. Local mate competition, variable fecundity, and information utilization in a parasitoid. *Anim. Behav.*
- FRANK, S. A. 1984. The behaviour and morphology of the fig wasps *Pegoscapus aseutus* and *P. jiminezi*: descriptions and suggested behaviors for phylogenetic studies. *Psyche* 91:289–307.
- . 1985. Hierarchical selection theory and sex ratios. II. On applying the theory, and a test with fig wasps. *Evolution* 39:949–964.
- . 1986. Hierarchical selection theory and sex ratios. I. General solution for structured populations. *Theor. Popul. Biol.* 29:312–342.
- GARLAND, T., JR., P. H. HARVEY, AND A. R. IVES. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41:18–32.
- GIBSON, J. B., AND B. P. BRADLEY. 1974. Stabilizing selection in constant and fluctuating environments. *Heredity* 33:293–302.
- GODFRAY, H. C. J. 1988. Virginity in haplodiploid populations: a study on fig wasps. *Ecol. Entomol.* 13:283–291.
- . 1990. The causes and consequences of constrained sex allocation in haplodiploid animals. *J. Evol. Biol.* 3:3–17.
- GRAFEN, A. 1989. The phylogenetic regression. *Philo. Trans. R. Soc. Lond. B Biol. Sci.* 326:119–156.
- GREEFF, J. M., AND S. G. COMPTON. 1996. Sequential oviposition and optimal sex ratios in pollinating fig wasps. *Ecol. Entomol.* 21:300–302.
- GREEN, R. F., G. GORDH, AND B. HAWKINS. 1982. Precise sex ratios in highly inbred parasitic wasps. *Am. Nat.* 120:653–665.
- GRIFFITHS, N. T., AND H. C. J. GODFRAY. 1988. Local mate competition, sex ratio and clutch size in bethylid wasps. *Behav. Ecol. Sociobiol.* 22:211–217.
- HAMILTON, W. D. 1967. Extraordinary sex ratios. *Science* 156:477–488.
- . 1979. Wingless and fighting males in fig wasps and other insects. Pp. 167–220 in M. S. Blum and N. A. Blum, eds. *Sexual selection and reproductive competition in insects*. Academic Press, London.
- HARDY, I. C. W. 1992. Non-binomial sex allocation and brood sex ratio variances in the parasitoid Hymenoptera. *Oikos* 65:143–158.
- HARDY, I. C. W., AND J. M. COOK. 1995. Brood sex ratio variance, developmental mortality and virginity in a gregarious parasitoid wasp. *Oecologia* 103:162–169.

- HARVEY, P. H., AND M. PAGEL. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford.
- HERRE, E. A. 1985. Sex ratio adjustment in fig wasps. *Science* 228: 896–898.
- . 1987. Optimality, plasticity, and selective regime in fig wasp sex ratios. *Nature* 329:627–629.
- . 1989. Coevolution of reproductive characteristics in twelve species of new world figs and their pollinator wasps. *Experientia* 45:637–647.
- . 1996. Implications of studies on a community of Panamanian figs: emerging patterns and future directions. *J. Biogeogr.* 23:593–607.
- HERRE, E. A., AND S. A. WEST. 1997. Conflict of interest in a mutualism: documenting the elusive fig wasp/seed trade-off. *Proc. R. Soc. Lond. B Biol. Sci.* 264:1501–1507.
- HERRE, E. A., C. A. MACHADO, E. BERMINGHAM, J. D. NASON, D. M. WINDSOR, S. S. MACCAFFERTY, W. VAN HOUTEN, AND K. BACHMANN. 1996. Molecular phylogenies of figs and their pollinator wasps. *J. Biogeogr.* 23:521–530.
- HERRE, E. A., S. A. WEST, J. M. COOK, S. G. COMPTON, AND F. KJELLBERG. 1997. Fig wasp mating systems: pollinators and parasites, sex ratio adjustment and male polymorphism, population structure and its consequences. Pp. 226–239 in J. Choe and B. Crespi, eds. *Social competition and cooperation in insects and arachnids*. Vol. 1. The evolution of mating systems. Cambridge Univ. Press, Cambridge.
- KONDRASHOV, A. S., AND D. HOULE. 1994. Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B Biol. Sci.* 258: 221–227.
- KONDRASHOV, A. S., AND L. Y. YAMPOLSKY. 1996. High genetic variability under the balance between symmetric mutation and fluctuating stabilizing selection. *Genet. Res.* 68:157–164.
- LANDE, R. 1975. The maintenance of genetic variability by mutation in a polygenic character with linked loci. *Genet. Res.* 26: 221–236.
- LANDE, R., AND S. J. ARNOLD. 1983. The measurement of selection on correlated characters. *Evolution*. 37:1210–1226.
- LEIGH, E. G., JR. 1990. Afterword. In J. B. S. Haldane. *The causes of evolution*. Princeton Univ. Press, Princeton, NJ.
- LYTHGOE, K. A. 1997. Consequences of gene flow in spatially structured populations. *Genet. Res.* 69:49–60.
- MACHADO, C. A., E. A. HERRE, AND E. BERMINGHAM. 1996. Molecular phylogenies of fig pollinating and non-pollinating wasps and the implications for the origin and evolution of the fig-fig wasp mutualism. *J. Biogeogr.* 23:531–542.
- MCCULLAGH, P., AND J. A. NELDER. 1989. *Generalized linear models*. Chapman and Hall, London.
- MORGAN, D. J. W., AND J. M. COOK. 1994. Extremely precise sex ratios in small clutches of a bethylid wasp. *Oikos* 71:423–430.
- NAGELKERKE, C. J. 1996. Discrete clutch sizes, local mate competition, and the evolution of precise sex allocation. *Theor. Popul. Biol.* 49:314–343.
- NAGELKERKE, C. J., AND I. C. W. HARDY. 1994. The influence of developmental mortality on optimal sex allocation under local mate competition. *Behav. Ecol.* 5:401–411.
- NASON, J. D., E. A. HERRE, AND J. L. HAMRICK. 1996. Paternity analysis of the breeding structure of strangler fig populations: evidence for substantial long-distance wasp dispersal. *J. Biogeogr.* 23:501–512.
- ORZACK, S. H., E. D. PARKER, AND J. GLADSTONE. 1991. The comparative biology of genetic variation for conditional sex ratio behaviour in a parasitic wasp, *Nasonia vitripennis*. *Genetics* 127: 583–599.
- PAGEL, M. D. 1992. A method for the analysis of comparative data. *J. Theor. Biol.* 156:431–442.
- . 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. *J. Theor. Biol.* 164:191–205.
- PHILLIPS, P. C. 1996. Maintenance of polygenic variation via a migration-selection balance under uniform selection. *Evolution* 50:1334–1339.
- PICKERING, J. 1980. Larval competition and brood sex ratios in the gregarious parasitoid *Pachysomoides stupidus*. *Nature* 283:291–292.
- POMIANKOWSKI, A., AND A. P. MØLLER. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B Biol. Sci.* 260:21–29.
- PURVIS, A., AND A. RAMBAUT. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *CABIOS* 11:247–251.
- ROSENHEIM, J. A. 1993. Single-sex broods and the evolution of nonsiblicidal parasitoid wasps. *Am. Nat.* 141:90–104.
- SCHARLOO, W., M. S. HOOGMOED, AND A. TER KUILE. 1967. Stabilizing and disrupting selection on a mutant character in *Drosophila*. I. The phenotypic variance and its components. *Genetics* 56:709–726.
- STEARNS, S. C., AND T. J. KAWECKI. 1994. Fitness sensitivity and the canalization of life history traits. *Evolution* 48:1438–1450.
- STEARNS, S. C., M. KAISER, AND T. J. KAWECKI. 1995. The different genetic and environmental canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* 8:539–557.
- TAYLOR, P. D. 1981. Intra-sex and inter-sex sibling interactions as sex ratio determinants. *Nature* 291:64–66.
- TURELLI, M. 1984. Heritable genetic variation via mutation-selection balance: Lerch's zeta meets the abdominal bristle. *Theor. Popul. Biol.* 25:138–193.
- TURELLI, M., AND N. H. BARTON. 1994. Genetic and statistical analyses of strong selection on polygenic traits: what, me normal? *Genetics* 138:913–941.
- WERREN, J. H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science* 208:1157–1159.
- WEST, S. A., AND E. A. HERRE. 1994. The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig-pollinator mutualism. *Proc. R. Soc. Lond. B Biol. Sci.* 258:67–72.
- WEST, S. A., E. A. HERRE, D. W. WINDSOR, AND P. R. S. GREEN. 1996. The ecology and evolution of the New World non-pollinating fig wasp communities. *J. Biogeogr.* 23:447–458.
- WEST, S. A., E. A. HERRE, S. G. COMPTON, H. C. J. GODFRAY, AND J. M. COOK. 1997. A comparative study of virginity in fig wasps. *Anim. Behav.* 54:437–450.
- WEST, S. A., S. G. COMPTON, S. L. VINCENT, E. A. HERRE, AND J. M. COOK. 1998. Virginity in haplodiploid populations: a comparison of estimation methods. *Ecol. Entomol.* 23:207–210.
- WIEBES, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annu. Rev. Ecol. Syst.* 10:1–12.
- . 1995. Agaonidae (Hymenoptera Chalcidoidea) and *Ficus* (Moraceae): fig wasps and their figs, xv (Meso-American *Pegoscapus*). *Proc. Kon. Ned. Akad. v. Wetensch* 98:647–653.
- WILLIAMS, G. C. 1979. The question of adaptive sex ratio in outcrossed vertebrates. *Proc. R. Soc. Lond. B Biol. Sci.* 205:567–580.
- WINDSOR, D. M., D. W. MORRISON, M. A. ESTRIBI, AND B. DE LEON. 1989. Phenology of fruit and leaf production by "strangler" figs on Barro Colorado Island, Panama. *Experientia* 45:647–653.

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APPENDIX

Assume that the fitness of the first female to enter a fig fruit (w_2) is described by equation (4). In this case, her optimal strategy is found by solving $\partial w_2/\partial m = 0$, which gives the equality

$$(1 - d) \left(\begin{aligned} & \left[\frac{-2(d^m - 1)(p - 1)}{2p(d^{m+m_2} - 1)(m^2 + 2mm_2 + m_2[m_2 - b])} \right] \\ & + \left[\frac{\log(d)(2d^m[m - b][p - 1] + pd^{m+m_2}[2m(m + m_2) - b(3m + m_2)])}{m + m_2} \right] \end{aligned} \right) = 0 \tag{A1}$$

and must be solved numerically. Sample relationships are shown in Figure 5.

The second differential of log fitness ($\partial^2 \log[w_2]/\partial m^2$) provides a measure of the strength of stabilizing selection, and equals

$$\begin{aligned} & \partial^2 \log(w_2)/\partial m^2 \\ & = \left(\begin{aligned} & - \left(\begin{aligned} & -2(d^m - 1)(p - 1) + 2p(d^{m+m_2} - 1) \left(1 - \frac{m_2 b}{[m + m_2]^2} \right) \\ & - 2d^m \log(d)(m - b)(p - 1) + \frac{pd^{m+m_2} \log(d)(2m[m + m_2] - b[3m + m_2])}{m + m_2} \end{aligned} \right)^2 \\ & + \left(2(b - m)(d^m - 1)(p - 1) + \frac{p(d^{m+m_2} - 1)(2m[m + m_2] - b[3m + m_2])}{m + m_2} \right) \\ & + \left(\begin{aligned} & \frac{4bpm_2(d^{m+m_2} - 1)}{(m + m_2)^3} - 4d^m \log(d)(p - 1) \\ & + 4pd^{m+m_2} \log(d) \left(1 - \frac{m_2 b}{[m + m_2]^2} \right) - 2d^m \log(d)^2 (m - b)(p - 1) \\ & + \frac{pd^{m+m_2} \log(d)^2 (2m[m + m_2] - b[3m + m_2])}{m + m_2} \end{aligned} \right) \end{aligned} \right) \\ & \left(2(b - m)(d^m - 1)(p - 1) + \frac{p(d^{m+m_2} - 1)(2m[m + m_2] - b[3m + m_2])}{m + m_2} \right) \end{aligned} \tag{A2}$$

Example relationships, numerically obtained by solving equation (A1) and then substituting it into equation (A2) are given in Figure 6.