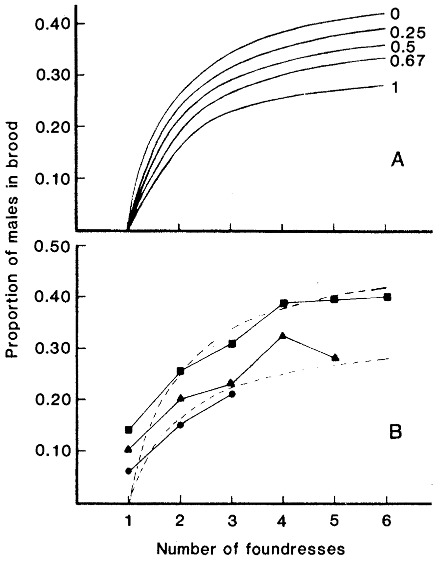
# Sex Ratio Adjustment in Fig Wasps

Abstract. *The effects of inbreeding and local mate competition have often been confounded in theoretical and empirical studies of sex ratio. A general model considering the interplay of their influences on sex ratio evolution is presented, and its predictions are compared with data collected from three fig wasp species. The results indicate that female fig wasps adjust the sex ratio of their offspring in response to both the intensity of proximate local mate competition and the level of inbreeding in the population.*

Sex ratio theory permits quantitative predictions of the outcome of natural selection to be made and tested. In a fundamental theoretical development, Fisher (*1*) showed that, in large randomly mating populations, frequency dependent selection will lead to equal parental investment in the two sexes and, generally, a 50:50 sex ratio. Later, Hamilton (*2*) pointed out that in many organisms mating often takes place between the offspring of one or a few foundress mothers in isolated subpopulations (broods) from which mated females disperse to found new broods and that under these conditions, local mate competition (LMC) should select for female biased sex ratios. Several studies suggest either a female bias in populations in which LMC effects are expected (*3–5*) or facultative adjustment of brood sex ratios by mothers in response to different intensities of LMC (*6–8*). However, the same subdivision of a mating population that leads to LMC also results in inbreeding, obscuring the roles of the two phenomena in sex ratio evolution. Some investigators deny any effect of inbreeding on sex ratio; and others use the two terms interchangably (*9–12*). I examined differences between the effects of LMC and inbreeding, developed a model considering the interaction of their influences on sex ratio in haplodiploid organisms, and tested the model's predictions with data collected from three species of fig pollinating wasps.

The evolution of female biased sex ratios due to LMC, originally conceived of as high interbrother competition for mates and later as differential group productivity (*3, 7, 9, 10*), does not depend on any effects of inbreeding (*13*). However, in inbreeding populations, mating among sibs leads to increased mother-offspring relatedness. In diploid organisms this increase is symmetrical with respect to sons and daughters. In haplodiploid organisms, since the haploid males derive all of their genome from the unfertilized eggs of diploid mothers, only the relatedness of mothers to daughters increases with inbreeding. This asymmetry in relatedness can result in female biased sex ratios independently of any effects of LMC or differential group productivity (*14*). The effects of inbreeding account for the female biased discrepancy between the predictions of Hamilton's original formulation of LMC theory and his simulations for an "unbeatable" haplodiploid sex ratio (*2, 3*).



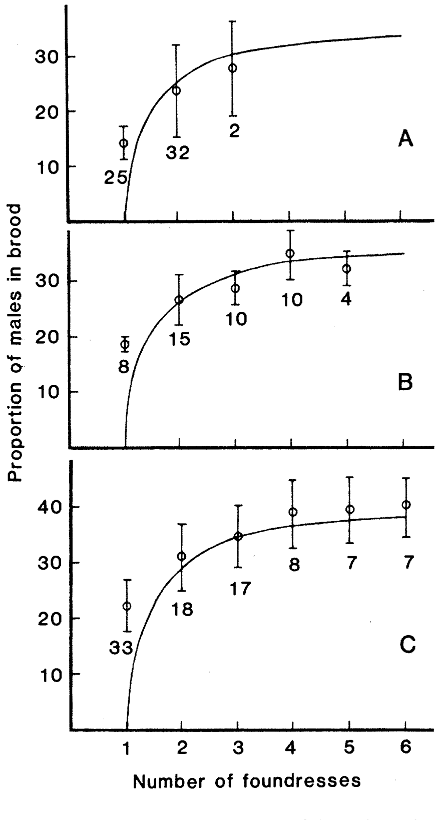


Fig. 1 (left). (A) Theoretical LMC sex ratios under different levels of inbreeding, expressed as a proportion of sib-mating ($1/n$) in a population. As inbreeding increases, the predicted brood sex ratios become more female biased. For one foundress, the predicted proportion of males is 0, although realistically enough males must be produced to ensure that all females are mated. (B) Means of empirical brood sex ratios for pollinator wasp species associated with *Ficus insipida* (■), *F. popenoei* (▲), and *F. citrifolia* (●) with theoretical curves (dashed lines) for high ($n = 1$) and low ($n = \infty$) levels of inbreeding for reference. Note that more inbred fig wasp species have more female biased brood sex ratios given the same number of foundresses than less inbred species. Fig. 2 (right). Comparisons of means and standard deviations of arcsine transformations of empirical brood sex ratios with theoretical curves generated for (A) *F. citrifolia* pollinating wasp (*Blastophaga* sp. 2) ($n = 1.13$), (B) *F. popenoei* wasp (*Blastophaga* sp. 1) ($n = 1.40$), and (C) *F. insipida* wasp (*Tetrapus costaricensis*) ($n = 1.99$). Sample sizes of broods sampled in each category are shown.

Previous models for predicting haplodiploid sex ratios under LMC have assumed a fixed level of inbreeding for a given intensity of LMC by considering only populations in which broods are all founded by a set number of mothers and, therefore, are all identical with respect to both LMC and inbreeding (*3, 4, 9*). However, if there is a distribution of foundress numbers within a population, the intensity of LMC (as judged by the number of foundresses contributing to a single brood) may vary among broods although the expected probability of being inbred is the same for all foundresses. Similarly, females from two populations may differ in the probability of being inbred and still found broods under the same intensity of LMC. Allowing inbreeding as well as LMC to vary permits the development of a more general theory with the additional prediction that in more inbred populations the response in brood sex ratio to a given intensity of LMC should be more female-biased. This prediction is formalized as

$$p = (1 - m)(2n - 1)/(4n - 1)$$

where $n$ is the harmonic mean number of foundresses per brood (as $n$ decreases, the overall population level of inbreeding through sib-mating increases) (*15*); $m$ is the proportion of individuals contributed to a given brood by a single mother, the reciprocal of the number of foundresses of a given brood (as $m$ increases, the effects of LMC that favor a more female bias sex ratio adjustment in that brood increase); and $p$ is the expected sex ratio (proportion of males) in the brood (*14*) (Fig. 1A).

The natural history of fig pollinating wasps makes them well suited for the examination of LMC and inbreeding effects on sex ratio (*16*). Some number of gravid, pollen-bearing foundress wasps enter a fig synconium (an enclosed inflorescence which ultimately develops into the ripe fruit), nearly simultaneously, pollinate the flowers, lay eggs, and die. As the fruit ripens, the wasp offspring mature, eclose, and mate inside. The winged females gather pollen, leave the synconium, and disperse to begin the cycle anew. The males are wingless and do not mate outside the synconium in which they are born. There is generally a one-to-one correspondence between pollinating wasp species and host fig species (*17–19*).

The intensity of LMC can be determined for the brood in any given synconium by counting the number of foundresses. As the number of foundresses decreases, $m$ (the reciprocal of foundress number) and LMC increase for that brood. The level of inbreeding in a wasp population can be estimated by sampling the distribution of foundress numbers among broods. Fig wasp species with lower harmonic mean foundress numbers will be more inbred (*14*). Therefore, both the effects of different intensities of LMC for a given level of inbreeding (within species comparisons of broods with different numbers of foundresses) and the effects of different levels of inbreeding for a given intensity of LMC (between species comparisons of broods with the same number of foundresses) on sex ratio can be examined.

Randomly sampling 20 synconia per fruit crop, I found the harmonic mean foundress number ($n$) to be 1.99 from 27 fruit crops of *Ficus insipida*, 1.40 from 6 crops of *F. popenoei*, and 1.13 from 8 crops of *F. citrifolia*, all sampled in the vicinity of Barro Colorado Island, Republic of Panama. The distribution of foundress numbers in each species was different from each of the others (Mann-Whitney test, $P < 0.02$). This indicates that the population of the wasp species associated with *F. insipida* (*Tetrapus costaricensis*) is less inbred than that of *F. popenoei* (*Blastophaga* sp. 1) which in turn is less inbred than that of *F. citrifolia* (*Blastophaga* sp. 2).

In each of the three fig wasp species the brood sex ratio rises with increasing foundress number, indicating a facultative adjustment according to the intensity of LMC. The magnitude of these adjustments is consistent with the model's predictions generated with the inbreeding estimates for each species (Fig. 2). Between species comparisons show that the sex ratio adjustment for a given intensity of LMC (number of foundresses) is more female biased in more inbred species (*20*) (Fig. 1B). Thus, the model's predictions of increasingly female-biased broods with both increasing LMC and inbreeding are met.

The data and the model constitute a confirmation and extension of natural selection and sex ratio theory. It is of particular interest to note that inbreeding acts to reduce the competition for mates both among male siblings and among sons of different mothers. If systematic reduction of competition is conducive to the evolution of cooperation and, potentially, social behavior, these data lend strength to the proposition that increasingly inbred population structures can promote the evolution of higher levels of sociality (*21*).

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14. The proportion of sib-mating in a population determines the equilibrium level of inbreeding, which here will be taken as the probability that an allele at a given locus that a daughter receives from her father is identical by direct descent (idd) to alleles of her mother. In order to calculate that probability, consider the population of mated mothers to be composed of four types: $a_1$ is the proportion of mothers somatically homozygous, mated with idd sperm; $a_2$ is the proportion of mothers somatically homozygous, mated with unrelated sperm; $a_3$ is the proportion of mothers somatically heterozygous, mated with sperm possessing alleles (idd) to one of the two maternal alleles; and $a_4$ is the proportion of mothers somatically heterozygous, mated with nonrelated sperm. Given the following assumptions—(i) random mating within broods, (ii) no inbreeding depression, (iii) no relation among cofoundresses of a given brood, (iv) equal contribution of offspring at equal sex ratios among cofoundresses of a given brood, and (v) equal total of females dispersing from all broods (*16*)—then the equilibrium proportion of each maternal type is given in terms of the harmonic mean number of foundresses per brood (*15*), $n$, by the following system of recursion equations: $a_1 = (1/n)(a_1) + (1/4n)(a_3)$; $a_2 = [(n-1)/n](a_1) + [(2n-1)/4n](a_3)$; $a_3 = (1/n)(a_2) + (1/2n)(a_3) + (1/2n)(a_4)$; $a_4 = [(n-1)/n](a_2) + [(n-1)/2n](a_3) + [(2n-1)/2n](a_4)$; and $a_1 + a_2 + a_3 + a_4 = 1$. At equilibrium, the proportion of daughters with both somatic alleles shared (idd) with their mothers is $a_1 + a_3 = 1/(2n-1)$. The standard inbreeding coefficient is $F = a_1 + a_2 = 1/(4n-3)$. Therefore, at equilibrium, the average daughter shares $1 + 1/(2n-1) = 2n/(2n-1)$ alleles (idd) with her mother while a son shares one. Computer simulations show the calculation of level of inbreeding to be robust in the face of varying foundress numbers through time and wide departures from the assumptions of equal contribution at equal sex ratio to broods. Multiple matings also do not affect the equilibrium level of inbreeding. The inbreeding dependent relatedness of daughters, $2n/(2n-1)$, and that of sons, one, to mothers is then used to weight the relatedness of broods of grandprogeny produced by sib crosses, daughter outcrosses, and son outcrosses. The expected frequency of each of these types of mating events is calculated with the proportion and sex ratio of the offspring a given mother contributes to a brood and the proportion and sex ratio of the offspring that other mothers contribute to that brood. The weighted frequencies are summed and differentiated with respect to the sex ratio of the given mother's offspring, $p$. The maximum is determined, and solving for $p$ produces a general formula for predicting brood sex ratios that maximize a mother's efficiency in exploiting a brood as a resource for propagating her alleles, given any level of inbreeding in the population, any proportion of the brood that she contributes, and any sex ratio of the offspring of other mothers

$$p = \frac{\left[\frac{(4n-1)(1-m)q}{(2n-1)}\right]^{1/2} - \frac{(4n-1)(1-m)q}{(2n-1)}}{\frac{(4n-1)m}{2n-1)}} \qquad (1)$$

    where $p$ is the proportion of males in a given foundress' offspring, $m$ is the proportion of a given brood a given mother contributes, $q$ is the proportion of males in the rest of the brood, and $n$ is the harmonic mean foundress number in the population. The Hamilton and Taylor-Bulmer equations for predicting haplodiploid sex ratios (*3, 9*) are special cases of this equation, derivable by setting $m = 1/n$ and $q = p$ (that is, by assuming fixed inbreeding for fixed intensities of LMC). The Werren equation that can predict sex ratios given variable relative contributions to broods by two foundresses and that did not take into account effects of inbreeding (*6*) can be derived by setting $(4n-1)/(2n-1) = 2$ and dividing through by $(1-m)$. Setting $(4n - 1/(2n-1) = 2$ makes Eq. 1 appropriate for diploid organisms. By setting equal the contributions of all cofoundresses to any given brood, another equation for $p$ emerges

$$(1-m)(2n-1)/(4n-1) \qquad (2)$$

    This equation is used to generate the predicted sex ratios resulting from the interplay of the effects of inbreeding and LMC in fig wasps (Figs. 1A and 2). Notice that the best brood sex ratio, $p$, for inbred haplodiploid foundresses $[(2n-1)/(4n-1) < ½]$ is female-biased even in populations to which their contribution of offspring is vanishingly small (as $m$ approaches 0, that is, in large outbreeding populations). Although the condition of inbred mothers in outbred populations is unstable, this result points to the fact that inbreeding selects for female-biased sex ratios independently of any effects of LMC or differential group productivity, which has been verified by appropriate simulation.
15. Since all the matings in a brood of a single foundress are between sibs, as are one-half in a brood of two foundresses by chance, and so on, the reciprocal of the arithmetic mean gives a low estimate of sib-mating. The best estimate of sib-mating is given by the reciprocal of the harmonic mean of foundress numbers.
16. The natural history of fig wasps justifies the assumptions of the model. Analysis of foundress distributions in the species studied support random distribution of foundresses among broods (synconia) (that is, there is no evidence of foundresses preferring to raise offspring in synconia with other foundresses). Also, since some species of figs are pollinated only by one foundress (complete inbreeding), inbreeding depression may not be important and random mating within broods is expected. Since any given fruit crop is composed of thousands of synconia and the distances between crops are large, the chance that two related females born in the same synconium will disperse to the same synconium in another crop is negligible. Cofoundress wasps enter each synconium at the same time and so contribute roughly equally to broods.
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20. Analysis of variance tests on the data show

foundress number, the square of foundress number, and calculated levels of inbreeding for each species to be significantly correlated to brood sex ratio in the expected pattern ($P < 0.001$). For foundress numbers greater than one, the theoretical curves lie within the 99 percent confidence intervals in all species. Differences among crops within species, total number of pollinator-parasitizing wasps, and total number of pollinator wasps reared per brood are not significantly correlated with the sex ratio in the sampled broods. Thus, sex-dependent mortality through parasitism and sex ratio skewing in response to resource availability do not account for the observed patterns.

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22. I thank D. M. Windsor who made this work possible, S. J. Wright who was instrumental in the development of the model, M. D. Rausher for statistical help, R. B. Foster for *Ficus* identification, J. T. Wiekes for *T. costaricensis* identification, L. F. Reid, D. E. Wheeler, M. J. Ryan, D. M. Feener, S. W. Skinner, S. E. Via, E. G. Leigh, Jr., A. R. Kiester, R. Lande, M. J. Wade, D. W. Schemske, T. P. Young, R. K. Colwell, D. S. Webster, H. F. Howe, and W. D. Hamilton for useful suggestions or help in preparing the manuscript. Special thanks to M. Rabinowitz. Support was provided by the Smithsonian Tropical Research Institute through its Environmental Sciences Program and by the University of Iowa through its Teaching and Research Fellowship Program. Equation 2 was derived independently by both J. H. Werren and M. K. Uyenoyama (personal communications).

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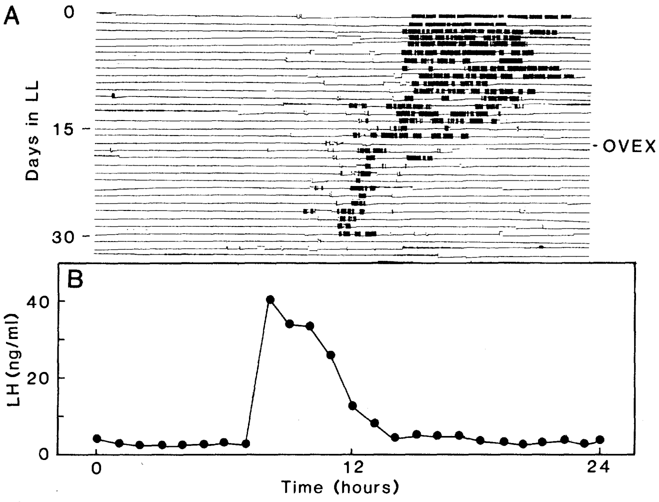
# Multiple Circadian Oscillators Regulate the Timing of Behavioral and Endocrine Rhythms in Female Golden Hamsters

Abstract. *A single daily "surge" in pituitary luteinizing hormone release was observed in ovariectomized–estrogen-treated hamsters expressing an intact circadian rhythm of locomotor activity. In contrast, two luteinizing hormone surges occurred within a single 24-hour period in hamsters whose activity rhythm had dissociated or "split" into two distinct components. These observations indicate that both behavioral and endocrine circadian rhythms are regulated by the same multioscillator system, which seems to be composed of at least two distinct circadian oscillators.*

Circadian rhythms are endogenously generated biological oscillations with periods of approximately 24 hours (*1*). These rhythms have been observed in biochemical, physiological, and behavioral events in both unicellular and multicellular organisms. That the circadian system in mammals is composed of multiple circadian oscillators (*2*) is supported by the finding that during prolonged exposure to constant light the rhythm of locomotor activity in rodents can dissociate or "split" into two distinct circadian components (*3*). During the early stages of splitting, the two activity components often show different free-running periods for several cycles before becoming stably synchronized to each other, with the two activity onsets occuring about 12 hours apart. The simplest explanation for this phenomenon is that the rhythm of activity is regulated by two mutually coupled oscillators, or sets of oscillators, each of which regulates one activity component (*3*, *4*).

Although splitting of circadian rhythms into two components has been observed in many vertebrate species, all clear cases of splitting have been of either the rhythm of locomotor activity or behavioral rhythms (such as feeding and drinking) that depend on the activity-rest state of the animal for their expression (*3*, *5*). It is not clear if splitting is a general property of vertebrate circadian rhythms or if it is related only to the activity-rest cycle of the animal. To determine whether nonbehavioral rhythms can also dissociate into two circadian components, we monitored the timing of the "surge" of luteinizing hormone (LH) in ovariectomized hamsters treated with estrogen (OVEX-E) and having either an intact or a split rhythm of locomotor activity. A surge in pituitary LH release occurs daily in ovariectomized hamsters after treatment with estrogen. In contrast, a single preovulatory LH surge occurs once every 4 days in hamsters with ovaries (*6*).



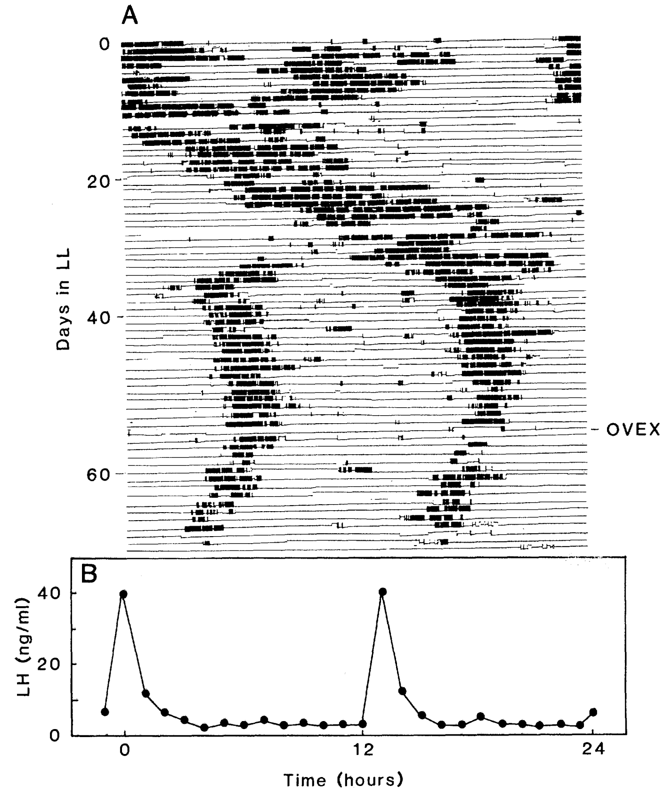


Fig. 1 (left). Continuous record of wheel running activity (A) and serum LH concentrations (B) of a representative OVEX-E hamster whose activity rhythm remained intact during exposure to constant light. The LH profiles are plotted so that each hourly sample corresponds to the time of sampling on the last day of each chart. Peak LH concentration was seen 1 to 4 hours before the onset of activity in all six animals with intact activity rhythms. Fig. 2 (right). Continuous record of wheel running activity (A) and serum LH concentrations (B) of a representative OVEX-E hamster whose activity rhythm had split into two components during exposure to constant light. Peak LH concentration occurred 0 to 4 hours before the onset of an activity bout in all ten animals with split activity rhythms.