ARTICLES

Sex allocation and clutch size in parasitoid wasps that produce single-sex broods

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The parasitoid wasp genus *Achrysocharoides* (Eulophidae) is unusual in that many of its species lay male and female eggs in single-sex clutches. The average clutch size of female broods is always greater than that of male broods, and in some species male clutch size is always one. We constructed models that predicted that severely egg-limited wasps should produce equal numbers of male and female eggs while severely host-limited wasps should produce equal numbers of male and female broods (and hence an overall female-biased sex ratio). Theory is developed to predict clutch size and sex ratio across the complete spectrum of host and egg limitation. A comparison of 19 surveys of clutch composition in seven species of *Achrysocharoides* showed a general pattern of equal numbers of male and female broods with a female-biased sex ratio (suggesting host limitation) although with considerable heterogeneity amongst collections and with a number of cases of unexpectedly low frequencies of male broods. Using a previous estimate of the relationship between fitness and size in the field, we predicted the maximally productive (Lack) clutch size for female broods of *Achrysocharoides zwoelferi* to be three. Of clutches observed in nature, 95% were equal to or smaller in size than the predicted Lack clutch size. When we manipulated local host density in the field, and as predicted by our models, clutch size and the proportion of female broods of *A. zwoelferi* decreased as hosts became more common, but the absolute frequency of male clutches was lower than expected.

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Much of life-history ecology involves the study of how animals and plants allocate limiting resources to competing somatic and reproductive functions (Stearns 1992). In different circumstances, or at different times, the nature of the limiting resource may change leading to selection for flexible life-history strategies. Parasitoid wasps are a good system for studying how animals respond to changes in limiting resources. Female wasps search the environment for hosts, on which they lay one or more eggs. Once hatched, the larvae develop by feeding on the host which has to provide all the necessary resources for growth until pupation. On finding a host, the searching female must make a series of often interrelated behavioural decisions. These include how many eggs to lay

(clutch size), and what the clutch sex ratio should be (parasitoid wasps are haplodiploid and females are able to determine the sex of their offspring by whether they fertilize the egg). The optimum strategy depends on the degree to which the wasp is limited by the number of eggs it carries or the number of hosts it can locate (review in Godfray 1994). It is normally difficult to identify the factor or factors limiting reproductive success (Driessen & Hemerik 1992) but in this paper we work with a group of parasitoid wasps with an unusual biology that allows the limiting factors to be identified indirectly.

The wasps we study belong to the genus *Achrysocharoides* (Hymenoptera, Chalcidoidea, Eulophidae). Different species of these insects display an unusual variety of reproductive strategies including the production of (1) small mixed broods of males and females; (2) small single-sex broods containing only males or females; (3) single-egg broods of males and small broods of females; and (4) species with thelytokous (parthenogenetic) reproduction (Askew & Ruse 1974; Bryan 1983). We are

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concerned here with species in categories 2 and 3: those in which male and female progeny are placed in single-sex broods. Most of our experimental work concerns one species, *A. zwoelferi* Delucchi, a category 3 species that produces single-egg clutches of males and small but variable clutches of females. At present we do not know why male and female offspring are segregated (but see Pickering 1980 and Rosenheim 1993), or why in category 3 species such as *A. zwoelferi* male clutch size is always one. In what follows, we treat this phenomenon as a constraint and consider the optimum sex ratio (expressed throughout this paper as the fraction of males), female clutch size and male clutch size (for category 2 species) in wasps that experience different degrees of egg and host limitation.

The first part of the paper is theoretical and consists of a model that predicts the change in sex ratio and clutch size for different regimes of egg and host limitation. The strategy of wasps at either end of the continuum has been discussed previously (Godfray 1994). Consider first purely host-limited wasps; in this case the factor limiting wasp reproductive success is hosts. Fisher's (1930) sex ratio principle states that other things being equal (see Frank 1990 for a detailed discussion of what this means) an organism should invest equally in male and female production. Where hosts are limiting and where sexes are segregated, this leads to equal numbers of male and female clutches. If the optimum single-sex clutch sizes differ, a biased sex ratio results. Now consider purely egg-limited wasps; in this case egg supplies limit reproductive success and the wasp should produce an equal number of male and female eggs (assuming these are equally costly to produce) and hence an unbiased sex ratio. In the theoretical section we predict how sex ratio and clutch vary between the ends of the egg/host limitation continuum by making assumptions about clutch size competition in *Achrysocharoides* that match what we observe in the field. In the first experimental section we survey the sex ratio and clutch composition of seven species of *Achrysocharoides* to find out which type of resource limitation is most consistent with the data.

The optimal clutch size also depends on whether the female wasp is limited by hosts or eggs. In the case of pure host limitation, the female should maximize its fitness returns per clutch and produce what has been called the Lack clutch size (Charnov & Skinner 1984, 1985) after Lack's (1947) argument that birds are selected to maximize their fitness gains per clutch. If the wasp is purely egg limited, it should produce the clutch size that maximizes offspring (as opposed to brood) fitness which will be one if offspring fitness declines monotonically with clutch size (Godfray 1987; Hardy et al. 1992; Vet et al. 1994; Wilson & Lessells 1994). There have been a number of attempts to estimate the Lack clutch size in parasitoids (reviewed in Godfray 1994), but these have been beset by the problem that laboratory estimates of the relationship between fitness and body size are likely to have underestimated the negative consequences of being small. In a previous study, we obtained a field estimate of the relationship between fitness and body size for female *A. zwoelferi* (West et al. 1996). In the second experimental section we measure the size of females developing in broods of different size, and couple this with the above estimate of size-specific fitness to predict the Lack clutch size. The prediction is compared with the distribution of clutch sizes observed in the field.

In the theoretical section, we show how in species with obligatorily single-egg clutches of males (category 3), the optimum female clutch size increases, and the optimum proportion of male broods decreases, as the wasp moves towards severe host limitation. One of the most likely correlates of the degree of host limitation is the abundance of hosts in the environment and we thus predict that females should lay larger clutches when hosts are scarce. In the third experimental section we report tests of these hypotheses involving manipulation of the local host density in the field.

BACKGROUND BIOLOGY

The genus *Achrysocharoides* (=*Enaysma*) is a member of the chalcidoid family Eulophidae in the subfamily Entedoninae (Bryan 1980a; Hanson 1983). The species it contains are facultatively gregarious endoparasitoids of leafmining larvae, almost exclusively of *Phyllonorycter* Hübner (Lepidoptera, Gracillariidae) (Askew & Ruse 1974; Bryan 1980a). *Achrysocharoides* females oviposit in first, second or third instar *Phyllonorycter* larvae after first causing temporary paralysis (Bryan 1980b). The final instar parasitoid larvae kill the host in its fourth or fifth instar and pupate in the mine (Bryan 1983). Most British *Achrysocharoides* species and their *Phyllonorycter* hosts are bivoltine (Bryan 1983). The eggs of the first generation of both host and parasitoid are laid in the spring and emerge as adults in the summer, while the eggs of the second generation are laid in the summer. Both host and parasitoid overwinter as pupae in mines within fallen leaves, emerging as adults the next spring. In the U.K., seven species or forms of *Achrysocharoides* produce single-sex broods: *A. butus* (Walker), *A. cilla* (Walker) *A. latreillii* (Curtis), *A. niveipes* (Thomson), *A. splendens* (Delucchi) and *A. splendens* form B (sensu Bryan 1980a, almost certainly a different species) and *A. zwoelferi* (Delucchi). Much less is known about *Achrysocharoides* in other parts of the world although species with single-sex broods occur in California, U.S.A. (J. Rosenheim & P. Ode, personal communication) and Japan (H. Sato, personal communication).

Achrysocharoides zwoelferi is a small wasp, approximately 2 mm in length, with a metallic blue-green thorax, blue abdomen (with a white dorsal spot in the male) and white legs. It is a parasitoid of *Phyllonorycter* larvae on *Salix* spp. (Askew & Ruse 1974; Bryan 1980a). In our main study site, Silwood Park (Berkshire, U.K.), the wasp is the most common parasitoid attacking *Phyllonorycter salicicolella* Sircom on *Salix cinerea* L. and *Salix caprea* L. Both host and parasitoid have two generations a year with the peak wasp flight periods in June and September.

PREDICTING REPRODUCTIVE STRATEGIES WITH SINGLE-SEX BROODS

Our aim in this section and the Appendix is to predict how the extent of egg or host limitation affects the optimal clutch size and sex ratio strategy of female parasitoid wasps that lay single-sex broods.

Consider a wasp that always lays males and females in separate clutches. We begin by assuming that the wasp is born with its complete lifetime supply of exactly x eggs (pro-ovigeny) and is invariably mated before beginning oviposition. Furthermore, we assume panmictic mating and that all hosts are of identical quality. Let wasps encounter hosts at a constant rate λ and suffer a constant risk of mortality m. We assume that the fitness of an adult female is some function $f(c)$ of the clutch size (c) within which it developed and similarly $g(d)$ is the fitness of an adult male that developed in a clutch of size d. We assume that the two functions are monotonically decreasing and hence that an individual offspring has its highest fitness in a clutch size of one. The fraction of hosts on which male eggs are laid is called p.

In the Appendix we show that the optimal female clutch size (c), male clutch size (d; when not fixed at one) and the proportion of male broods (p) are a function of the severity of within-clutch competition and a single dimensionless parameter combination, $\phi = \mu x/\lambda$ which summarizes the adult life history. In the case of pure egg limitation, ϕ approaches zero; while for pure host limitation, ϕ approaches infinity. Host limitation is greatest when the risk of mortality is high, initial egg load is high, and host encounter rate is low. We construct an index of host limitation $\Phi = \phi/(1+\phi)$ that maps the egg/host limitation continuum on to the unit interval.

We illustrate the relationship between the wasp's optimal reproductive strategy and the degree of host limitation for two cases. (1) Category 2 species where wasps produce single-sex clutches of variable size. We assume that the fitness consequences for males of developing in large clutches are more severe than those for females, leading to the optimal size of male broods being less than that of female broods, the pattern observed in the field. (2) Category 3 species where wasps produce variable-sized female broods but males are always laid in clutches of one. In the Appendix we show that a model for synovigenic wasps, which in contrast to pro-ovigenic wasps mature eggs throughout their life, gives very similar results. We thus present results for only the pro-ovigenic case.

Figure 1a, b shows how the optimal clutch size varies with the index of host limitation. Because we assume wasp larvae have highest fitness when developing alone in the host, severely egg-limited wasps always produce clutch sizes of one. As host limitation becomes more severe, the number of eggs laid rises to the Lack clutch size. In Fig. 1c, d the corresponding plots for the optimal sex ratio are illustrated. Egg-limited wasps always produce an equal sex ratio but this becomes more female biased as host limitation increases in importance. For severely host-limited wasps, the optimum ratio of males to females equals the ratio of the male Lack clutch size to the female Lack clutch size (i.e. the ratio of maximally productive pure male and pure female clutches). If we had assumed that females suffered more than males from competition within the host (which appears not to happen in *Achrysocharoides*), then increasing host limitation would be associated with a more male-biased sex ratio. Finally, Fig. 1e, f, shows the optimal proportion of male broods as a function of host limitation. At either end of the spectrum, equal numbers of male and female broods are predicted, but for intermediate values, where females are partially egg- and partially host-limited an excess of male broods is expected. The result arises from the conflicting selection pressures on the wasp to produce more of the rarer sex (to maximize fitness through competition for mates) while still using hosts efficiently by producing larger clutches in female than male broods.

Both the pro-ovigenic and synovigenic models make the simplifying assumption that the strategy of the wasp is uninfluenced by its current state. More sophisticated dynamic optimization models could be constructed to predict how the strategy of the wasp varies in response to changing egg load, or changing host distribution (Mangel 1989a, b), but the added complexity of these models is probably not warranted at present by the extent of our information about the wasps' behaviour in the field.

METHODS

Cross-species Comparison of Brood Ratios

To examine natural variation in clutch size and sex ratio in seven species of *Achrysocharoides*, we used (1) data published by Bryan (1983); (2) our data on *A. zwoelferi* (see below); (3) data on four species reared by A. Rott (personal communication); (4) data on *A. splendens* reared by S. Perry (personal communication). Bryan's data were collected largely in the north of England while all the other data were collected in Berkshire in the south of England, the majority from Silwood Park. In all cases mined leaves were collected from the field and reared in cork-stoppered glass vials in outside insectaries. We distinguish between collections made in the summer and autumn generations (except for Bryan's published data where information from the two generations was combined).

The Lack Clutch Size for Female Broods of *A. zwoelferi*

Here we predict the Lack clutch size for female broods of *A. zwoelferi* and compare it with data from the field. We measured the body size of females developing in broods of different size and used these data in conjunction with the relationship between adult size and fitness determined by West et al. (1996) to calculate the clutch size that provided the highest fitness returns for the parent.

We determined the effect of clutch size on female body size by measuring the hind tibia length (HTL) of *A. zwoelferi* females that had emerged from clutches of various size in naturally occurring mines. We collected
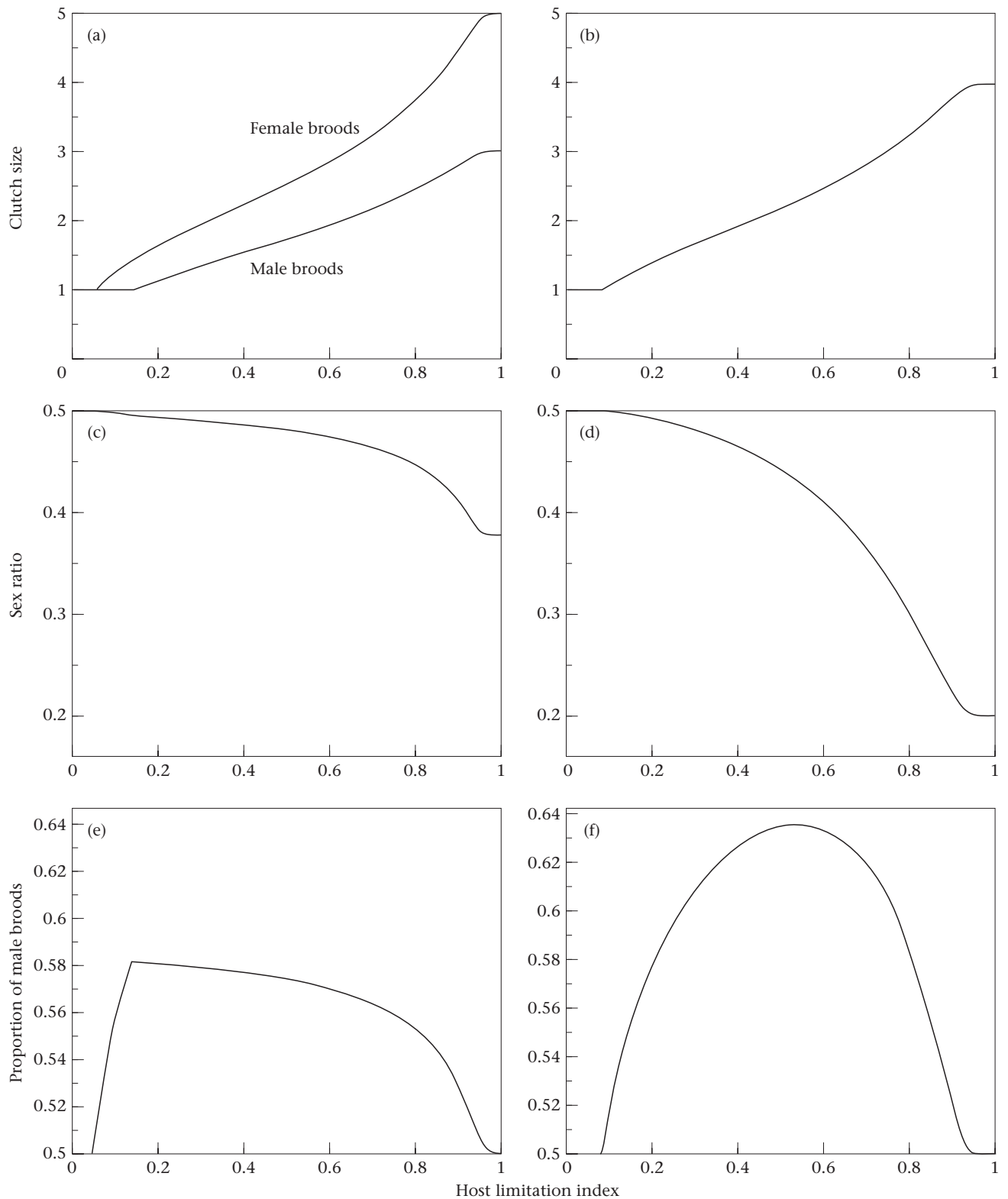
Figure 1. The predicted relationship between the index of host limitation and (a, b) clutch size, (c, d) sex ratio (proportion males) and (e, f) the proportion of male broods. In (a), (c), (e), both the male and female clutch size are allowed to vary (with $a=1/75$; $b=1/27$ where a and b are constants representing the rate at which fitness declines with increasing clutch size); in (b), (d), (f), the male clutch size is fixed at one (with $a=1/48$). Values of the parameters a and b were chosen to give clutch sizes of the order observed in *Achrysocharoides* species in the field. Note in (e), (f) the proportion of male broods can never be less than 0.5.

mature, first generation mines of *P. salicicolella* during June and July 1993 from an area of damp mixed woodland and grassland in Silwood Park, and placed them individually in corked glass tubes in an outside insectary. At the time of collection, the parasitoids had completed larval development and pupated. Mines were checked daily and the number and sex of emerging *A. zwoelferi* were recorded. The HTL of each wasp was then measured. In broods containing more than one female we used the average HTL to avoid pseudoreplication.

The form of the relationship between fitness and body size was obtained from the same population of wasps (West et al. 1996). We estimated it by comparing the size distributions of female wasps emerging from hosts and female wasps caught searching for hosts. Differences in these two distributions reflect size-dependent fitness, of which longevity is probably a major component. We fitted an exponential-polynomial equation to the data using maximum likelihood techniques to give the fitness relationship

$$\text{Female fitness} \propto \exp(a_2\,\text{HTL} + a_3\,\text{HTL}^2) \qquad (1)$$

with estimates for a_2 of 178.5 ± 67.1 ($\bar{X} \pm$ SE) and for a_3 of -248.7 ± 98.5. The equation allows us to measure only relative and not absolute fitness (i.e. the intercept a_1 cannot be estimated) although the former is all that is required to calculate the Lack clutch size.

Host Density Manipulation

In this experiment we manipulated host density, and hence indirectly the degree of host limitation, to see whether wasps laid smaller female clutches, and more nearly equal ratios of the two brood types, at high densities. We collected mature second generation mines of *P. salicicolella* at Silwood Park during June and July 1994, and placed them individually in corked glass tubes in an outside insectary. At the beginning of July 1994 we placed eight large muslin sleeves over branches of individual *Salix cinerea* at Silwood Park. These sleeves prevent access by both moths and parasitoids. During August 1994 we checked the collected mines daily for the emergence of adult moths, and placed between one and five individual females (and an equal number of males) in each sleeve to obtain a range of mine densities. After approximately 3 weeks the mines had developed within the sleeves to a size at which they could be parasitized by first generation females of *A. zwoelferi*. At this point we removed all the muslin sleeves simultaneously and recorded the density of mines per leaf in each cage, and measured the size (length and width) of each mine. One week after the muslin sleeves were removed, they were replaced to prevent further parasitoid attack, hence minimizing the risk of superparasitism. The muslin sleeves were on different trees and unlikely to have been visited by the same wasps. A muslin sleeve contained several hundred leaves. During September 1994 we picked all the mines that had completed development in the sleeves and stored them in an outside insectary, as described above.

The wasps and moths emerged from these collections during May and June 1995, whereupon we recorded the number and sex of any emerging *A. zwoelferi*. We repeated this experiment in the next generation of moths and parasitoids (the first generation in 1995), using 12 large muslin sleeves.

Statistical Analysis

Proportion data such as sex ratio or the fraction of male broods often have non-normally distributed errors and are hard to analyse with traditional ANOVA techniques. To avoid these problems we analysed the data using generalized linear modelling techniques (McCullagh & Nelder 1989) which are implemented in the GLIM statistical package (Crawley 1993). We assumed binomially distributed errors and employed a logit link function. Hypotheses were assessed using the chi-square test on differences in deviance. We checked the appropriateness of the assumption of binomial errors by comparing the residual deviance with the residual degrees of freedom after fitting the explanatory variable. Large relative values of the residual deviance indicate overdispersion which may result in overestimation of significance levels. For moderate levels of overdispersion, we rescaled the deviance by the Heterogeneity Factor (HF), the ratio of the residual deviance to the degrees of freedom (McCullagh & Nelder 1989; Collett 1991). For HF>3 we used either standard ANOVA after angular transformation or nonparametric techniques. Standard residual and normal quantile plots were used to assess the fit of the statistical model.

RESULTS

Cross-species Comparison of Brood Ratios

Table 1 summarizes the data for all 19 collections of seven species of *Achrysocharoides*. Altogether, there are records of 8358 wasps from 5518 separate broods. Across all species, 47% of broods contained only males while 33% of all emerging wasps were males. The combined data thus suggest that *Achrysocharoides* is nearer the host-limited than the egg-limited end of the resource spectrum. Clutch sizes tended to be smaller in autumn when the hosts are more abundant (Bryan 1983), consistent with the predictions of the model.

Host-limited wasps should produce equal numbers of male and female broods. We used a *G* test to look for biased ratios in the different samples, although note that this test assumes statistical independence amongst broods, an assumption that may be violated. Of the 19 collections, no significant deviations from equality were found in 12 cases although statistical power was low in three of these collections because of small sample size (Table 1). Where significant differences were observed, in five cases there was often a strong excess of females while in two cases a relative modest excess of males was found. Biased brood ratios were not associated with wasp generation or with species. However, there did appear to be an

Table 1. Summary of brood compositions in species of *Achrysocharoides* with single-sex broods

Species name	Generation*	Source†	Fraction single-sex broods‡	Male broods§		Female broods§		Sex ratio**	Brood ratio††	G test‡‡	P
				Number	Clutch size	Number	Clutch size				
butus	Both	Bryan	1.00	4	1.0	5	1.4	0.31	0.44	0.11	NS
cilla	Both	Bryan	0.99	406	1.2	445	1.9	0.35	0.48	1.54	NS
latreillii	Both	Bryan	1.00	112	1.0	111	2.0	0.29	0.50	0.02	NS
latreillii	Summer	Rott	1.00	164	1.4	189	1.9	0.39	0.46	1.77	NS
latreillii	Autumn	Rott	1.00	118	1.2	143	1.4	0.40	0.45	2.40	NS
niveipes	Both	Bryan	0.99	432	1.0	344	2.2	0.40	0.56	10.01	<0.01
niveipes	Summer	Rott	1.00	6	1.0	12	1.8	0.21	0.33	2.04	NS
niveipes	Autumn	Rott	1.00	28	1.0	67	1.7	0.20	0.29	16.49	<0.001
splendens	Both	Bryan	0.92	19	1.5	29	1.9	0.35	0.40	2.10	NS
splendens	Summer	Rott	1.00	40	1.0	85	2.0	0.19	0.32	16.57	<0.001
splendens	Autumn	Rott	1.00	25	1.0	30	2.0	0.30	0.45	0.46	NS
splendens	Summer	Perry	0.99	28	1.0	107	2.1	0.11	0.21	49.31	<0.001
splendens	Autumn	Perry	0.99	155	1.0	121	1.8	0.42	0.56	4.20	<0.05
splendens B	Both	Bryan	0.97	13	2.4	14	2.6	0.45	0.48	0.04	NS
zwoelferi	Both	Bryan	0.98	103	1.0	152	2.0	0.28	0.40	9.48	<0.01
zwoelferi	Summer	Rott	1.00	149	1.0	140	1.9	0.36	0.52	0.28	NS
zwoelferi	Autumn	Rott	1.00	204	1.0	320	1.4	0.31	0.39	25.89	<0.001
zwoelferi	Summer	West	1.00	283	1.0	298	2.1	0.31	0.49	0.39	NS
zwoelferi	Autumn	West	1.00	297	1.0	320	2.0	0.32	0.48	0.86	NS

*Summer or autumn generation or both combined.
†See Methods.
‡The fraction of broods from which only one sex was reared.
§The number of clutches collected and the mean clutch size are given.
**Proportion of males.
††Proportion of male broods.
‡‡Tests the hypothesis of equal numbers of male and female broods.

association with the source of the collection with samples collected by Rott and Perry tending to be more female biased than those by Bryan and by us.

We were surprised to find regional variation in whether males always developed in broods of one. In Bryan's collections from the north of England, *A. latreillii* (a *Quercus* specialist) was always solitary while male *A. splendens* (an *Alnus* specialist) developed in broods with average size 1.5. In our collections from Berkshire in the south of England *A. latreillii* males were found in broods of average size 1.3, while male *A. splendens* were always in broods of one.

The Lack Clutch Size for Female Broods of *A. zwoelferi*

Table 2 shows the hind tibia length of females emerging from clutches of different size. Females from small clutches were significantly larger (Spearman correlation coefficient: $r_S=0.18$, $N=106$, one-tailed $P<0.05$), a non-parametric statistic being used because Bartlett's tests showed significant ($P<0.001$) heteroscedasticity in both untransformed and logarithmically transformed data. Table 2 also gives the estimated fitness of individual females developing in broods of different size, and the net fitness returns to the mother from the whole clutch. In both cases, fitness is calculated using equation (1). Parental fitness per host appears to be maximized at a clutch size of three, although it should be noted that we have very little data for clutch sizes greater than three.

If three is the Lack clutch size for *A. zwoelferi*, broods containing up to and including that number of wasps should be observed, but not more. Figure 2 shows the distribution of female brood sizes in the experimental manipulation and in the field collections. The majority of clutches were smaller than the Lack clutch size, especially in the field collections. Overall, less than 5% of clutches exceeded the Lack clutch size.

Host Density Manipulation

The average size of female *A. zwoelferi* broods declined significantly with increasing host density in the sleeve ($F_{1,17}=33.77$, $P<0.01$; Fig. 3). Brood size was significantly larger in the autumn generation than in the summer generation ($F_{1,17}=7.05$, $P<0.05$) but

Table 2. The average hind tibia length and individual fitness of female *A. zwoelferi* developing in broods of different size, with the total fitness returns to the parent wasp

Brood size	Hind tibia length	Estimated individual fitness	Estimated brood fitness
1	0.337±0.005 (59)	1.07±0.01	1.07±0.01
2	0.332±0.005 (36)	1.01±0.01	2.02±0.01
3	0.321±0.005 (10)	0.86±0.03	2.57±0.07
4	0.290 (1)	0.37±0.10	1.49±0.34

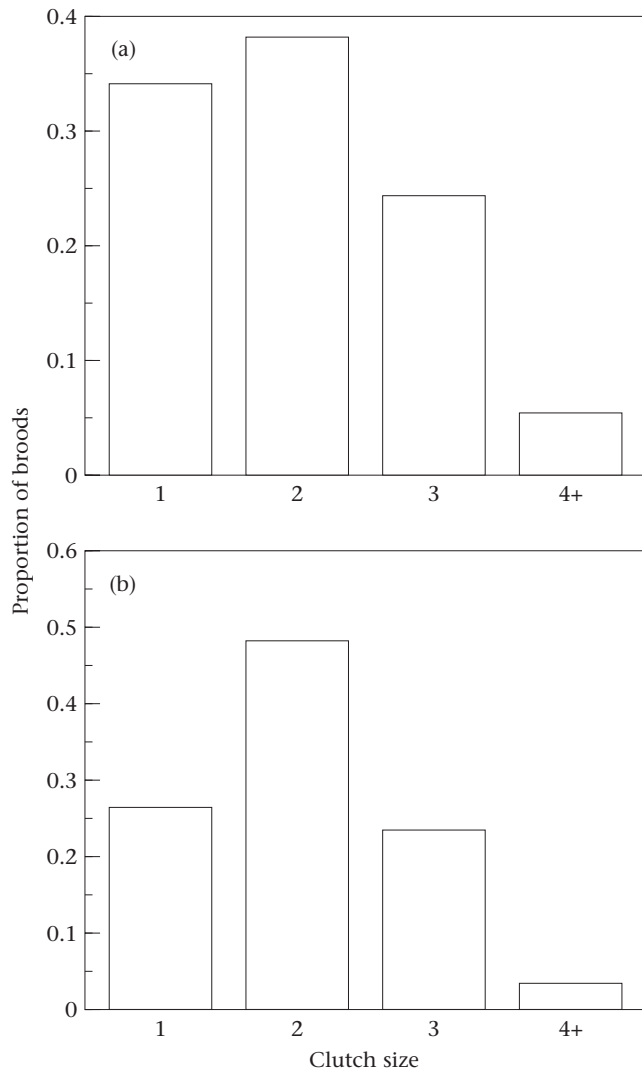Means are given±SE. Sample sizes are given in parentheses.

Figure 2. Distribution of female clutch sizes of *A. zwoelferi* from (a) the experiments in which local host density was manipulated and (b) the general field collections.

Figure 3. The relationship between clutch size and host density in the field manipulation experiments. Fitted lines are from the log-linear analysis. ◆, - - - -: Autumn generation; ○, ——: summer generation.

Figure 4. The relationship between the proportion of male broods and host density in the field manipulation experiments. ◆: Autumn generation; ○: summer generation. A common line was fitted to data from both generations.

there was no significant interaction between density and generation.

The proportion of male broods laid in a sleeve increased significantly with host density in that patch ($\chi_1^2 = 5.95$, $P < 0.025$, HF = 1.72, $N = 20$; Fig. 4). Neither the slope ($\chi_1^2 = 0.24$, $P > 0.1$) nor the intercept ($\chi_1^2 = 1.91$, $P > 0.1$) of this relationship differed significantly in the two generations.

DISCUSSION

Wasps in the genus *Achrysocharoides* offer an unusual opportunity to study factors limiting reproductive success in the field. For reasons that we do not currently understand, females of a majority of species lay single-sex clutches. Moreover, the clutch size of male broods is always lower than that of female broods, and in a number of species is one egg. We used simple models to predict the optimum reproductive strategy of female wasps across
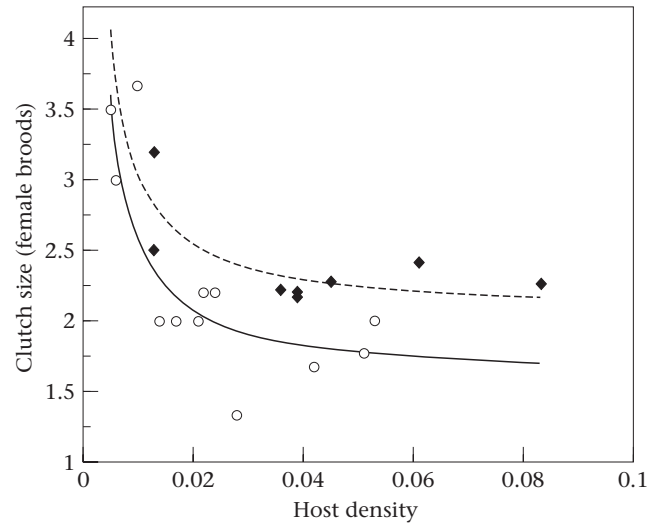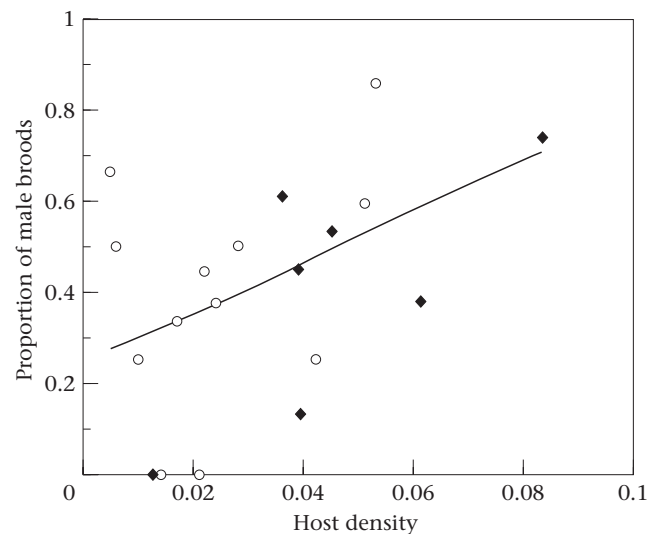
the continuum from severe egg limitation to severe host limitation. The models were based on the assumption that competition within the brood led to different-sized male and female clutches, and that in some circumstances male clutches were always one. This assumption that the optimum clutch size is determined by larval competition has not been tested for *Achrysocharoides*, although it is supported by a number of studies of other gregarious parasitoids (reviewed by Godfray 1994). Experiments in which we manipulated host density in the field provided support for several of our predictions. In addition, by observing whether females on average tend to lay equal numbers of male and female broods, or equal numbers of male and female eggs, we were able to

assess whether wasps on average tended to be egg- or host-limited in the field.

Theory suggests that severely egg-limited females should produce equal numbers of male and female eggs, and that host-limited females should produce equal numbers of male and female broods (and hence an overall female-biased sex ratio). Between these two extremes, females are predicted to produce an excess of male broods (and hence the sex ratio remains female biased). An excess of female broods and a male-biased sex ratio are never predicted. Our samples of *A. zwoelferi* contained equal numbers of male and female broods, with the sex ratio heavily biased towards females. We have also collated data collected by several workers on a number of *Achrysocharoides* species. These data must be treated with some caution as most were collected for different purposes. Overall, however, these data gave similar results: the number of male and female broods was near equality, and 14 out of 19 samples contained either an approximately equal number of male and female broods or a significant excess of male broods. The sex ratio was heavily biased towards females in all species (Table 1). However, there was considerable variation amongst samples, most of which we cannot explain, although some may be associated with differences in the procedures used by the different collectors.

Overall, these results provide qualitative support for our models, and argue for the importance of host limitation as the underlying cause of the observed female-biased sex ratios. However, a significant excess of female broods did occur in a minority (five) of collections. Apart from differences in collection methods there are a number of possible biological explanations for this. First, random mortality may lead to an underestimation of the number of male broods because the clutch size of male broods is less than that of female broods. Suppose males always develop in clutches of one and females in clutches of two, and that all individuals survive with probability p. The death of a single male leaves no trace but the death of a single female leaves a survivor and hence the brood is categorized as female. The ratio of observed male to female broods is thus $p:p(2-p)$. Estimates of the preadult mortality rate in parasitoids have given values of p from 0.23 to 0.93 (Nagelkerke & Hardy 1994), which would depress a predicted fraction of male broods of 0.5 to an observed 0.36 and 0.48, respectively. This effect will be accentuated if male mortality is higher than female mortality (as it might be in haplodiploid species, Smith & Shaw 1980), but reduced if mortality tends to destroy whole broods rather than individuals. Note that sex-differential mortality will not normally influence selection on the primary sex ratio.

Predicting the proportion of male broods also becomes more complicated if ovipositing wasps tend to place female broods in hosts of different size from those in which they lay male broods. Such 'conditional sex expression' strategies could occur in *Achrysocharoides* if broods of multiple females gain more from developing in large hosts than broods of single males (Charnov et al. 1981). In the presence of conditional sex expression, simple Fisherian predictions apply only approximately (Frank 1986, 1990) and there is a bias in primary sex allocation towards the sex that develops under the poorer conditions (i.e. here one would expect more male broods than female broods). However, the extent of the bias is hard to predict (Frank & Swingland 1988) and its deduction from secondary sex ratios is complicated by possible host-size-dependent mortality in the field, and during rearing in the laboratory. We are currently investigating the possibility that *A. zwoelferi* tends to lay female clutches on larger hosts.

Our models may also help explain the reproductive strategies of two other parasitoid species that segregate the sexes in different clutches (again for reasons that are not understood). We predict equal numbers of male and female broods or an excess of the sex produced in smaller clutches. Salt (1931) found that *Bracon terebella* (sensu lato), a gregarious parasitoid of the wheat-stem sawfly, *Cephus pygmeus*, produced 89% single-sex broods. The average clutch size of male broods (4.62) was significantly greater than that of female broods (3.34), and there was an excess of female broods ($G=43.91$, $N=249$, $P<0.001$). Godfray & Shaw (1987) studied *Eulophus ramicornis* (then called *E. larvarum*), a parasitoid of macrolepidopteran larvae, in which 82% of the summer generation broods studied were of a single sex. The average clutch sizes of male and female broods were not significantly different and equal numbers of male and female broods were produced ($G=0.33$, $N=27$, $P>0.1$).

The size of adult female *A. zwoelferi* is negatively correlated with the size of the brood in which they developed. For the first time for a gregarious parasitoid an estimate of the relationship between fitness and size in the field is available which allows us to predict the Lack clutch size. For female *A. zwoelferi*, the predicted Lack clutch size, and hence the predicted maximum clutch size that should be seen in the field, was three. The observed distribution of clutch sizes is largely in agreement with this prediction with over 95% of the clutches containing fewer than four wasps. However, a few broods had four or more individuals, and large clutches were also observed in the local density manipulation experiment in patches with very low host density (Fig. 3). The large clutches could indicate (1) that our predicted Lack clutch size is too low; (2) that wasps are using a more subtle strategy and assessing the quality of individual hosts (our prediction was based on averaged data); (3) occasional errors by the wasps or (4) instances of superparasitism.

Where do our data suggest *A. zwoelferi* lies on the host/egg limitation continuum? Strongly host-limited wasps should produce equal numbers of male and female broods, each of the Lack clutch size. We observed nearly equal numbers of male broods (580) and female broods (618; Table 1) which suggests strong host limitation, but typical female brood sizes were smaller than the Lack clutch size which suggests an intermediate position on the egg/host limitation continuum. Possible underestimation of the number of male broods for the reasons discussed above may explain this discrepancy. What can be said is that our data strongly argue against severe egg limitation. More specifically, our model allows us to

provide a quantitative estimate of the average host limitation index in the field based upon the observed distribution of brood sizes. Assuming a Lack clutch size of three we estimate that the host limitation index of *A. zwoelferi* is 0.68. Estimates for the other species can also be obtained, and give very similar values: 0.69 for *A. cilla*; 0.72 for *A. latreillii*; 0.74 for *A. niveipes*; and 0.72 for *A. splendens*.

The sleeve experiments showed that clutch size was influenced by local host density with smaller broods occurring when hosts were common. This result is predicted by both our models and by previous clutch size theory. Observational data on the correlation between clutch size and host density in the field have been reported before, and there are also a few laboratory experiments showing a relationship between encounter rate with hosts and the number of eggs laid (Jackson 1966; Ikawa & Suzuki 1982; see also review in Godfray 1994). However, we know of no other field experiment demonstrating this pattern.

Our models predict that the proportion of male broods should always be greater than 0.5 and should display a domed relationship with the index of host limitation (Fig. 1f). We cannot calculate the index directly, but the range of clutch sizes observed in the sleeve experiments was largely 50–75% of the predicted Lack clutch size, which is consistent with the index varying from about 0.5 to 1 as host density decreases. We thus expect the proportion of male broods to increase as hosts become more common, a pattern shown by our data (Fig. 4). However, male broods were substantially rarer than predicted, the proportion falling as low as 0.3 for the sleeves with the lowest host density. We cannot at present explain this observation, although relatively higher mortality of male clutches of one (see above) is a likely explanation.

To conclude, we suggest our work on *Achrysocharoides* is relevant to two more general issues in evolutionary ecology. First, it shows how life-history strategies are influenced by the factors limiting reproductive success. Both clutch size and sex ratio decisions depend strongly on whether the female wasp is limited by its egg supply or by its ability to find hosts. Second, it provides an example of how two aspects of reproductive strategy that are often studied separately, clutch size and sex ratio, are intimately connected. The optimal clutch size for *Achrysocharoides* is a function of optimal sex ratio and vice versa. How suites of traits evolve simultaneously is an important problem in current life-history theory (Stearns 1992; Rosenheim et al. 1996) and we argue that parasitoid wasps in general, and those with unusual biology such as *Achrysocharoides* in particular, may be valuable tools for disentangling the different actions of natural selection.

Acknowledgments

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APPENDIX

Here we construct two general models that predict how the extent of egg or host limitation affects the optimal clutch size and sex ratio strategy of females that lay single-sex broods. The first model assumes that the wasp is pro-ovigenic, born with a fixed supply of eggs, while the second model assumes a synovigenic wasp that matures eggs throughout its life. We also consider the special case of when the male clutch size is constrained to be one, but the female clutch size can vary. The methods follow Hunter & Godfray (1995) who studied sex allocation in autoparasitoid wasps.

Pro-ovigenic Model

Assume that females are born with x eggs, encounter hosts at a constant rate λ, and suffer a constant risk of mortality μ. Females search the environment and lay a clutch of d males in a fraction p of the hosts encountered, and a clutch of c females in the remaining fraction $(1-p)$ of hosts encountered. The fitness of a male ($g(d)$) or female ($f(c)$) is assumed to decrease with the size of the clutch in which they developed. We seek the values of p, c and d that maximize the reproductive success of a female.

Consider a rare mutant that adopts the strategy $\{p', c', d'\}$ in a population playing $\{p, c, d\}$. Its maximum reproductive life span, τ (i.e. how long before it runs out of eggs), is

$$\tau = \frac{x}{\lambda(p'd' + (1-p')c')} \tag{A1}$$

its initial egg load divided by its rate of oviposition (recall male and female clutch sizes are different). The mutant female's expected fitness is thus

$$W'(p',c',d') = \left[\int_0^\tau e^{-\mu t} dt \right] [\lambda(p'd'g(d')v \\ + (1-p')c'f(c'))], \tag{A2}$$

where the first term in square brackets is the wasp's expected life span and the second its rate of gain of fitness per unit time. The parameter v represents male mating success which, if the mutant is rare, equals the ratio of wild-type females and males in the environment,

$$v = \frac{(1-p)cf(c)}{pdg(d)}. \tag{A3}$$

The optimum strategy is found by simultaneously solving $\partial W'/\partial\theta'|_{\theta=\theta'}=0$ for $\theta=\{p, c, d\}$ which gives three equalities,

$$\exp\frac{\phi}{B} = 1 + \left[\frac{2\phi}{B^2} \right] \left[\frac{1}{A_\theta} \right]; \tag{A4}$$

where $\phi = \mu x/\lambda$, $B=c(1-p)+dp$ and $A_p=(1-2p)/[p(1-p)(d-c)]$; $A_c=[1/(1-p)][1/c-1/c_L]$ and $A_d=[1/p][1/d-1/d_L]$. The two terms c_L and d_L are the female and male Lack clutch sizes: $c_L=-f(c)/f'(c)$ and $d_L=-g(d)/g'(d)$. Note that the three adult wasp life-history parameters (μ, λ and x) enter only in the parameter combination ϕ. For severe host limitation, $\phi \to \infty$ and hence equation (A4) reduces to $A_\theta=0$ and $\{p, c, d\}=\{0.5, c_L, d_L\}$ as expected from a verbal argument (Godfray 1994).

Values for $\{p, c, d\}$ have to be obtained numerically after specifying the functions $f(c)$ and $g(d)$. We used the quadratic competition functions of Parker & Courtney (1984), $f(c)=1-ac^2$ and $g(d)=1-bd^2$ where a and b are the constants representing the rate at which fitness declines with increasing clutch size; although we obtained qualitatively identical results with the exponential forms of Waage & Godfray (1985). The solutions

when male clutch size is constrained to be one are found by putting $d=1$ and solving for p and c.

Synovigenic Model

Synovigenic wasps mature eggs throughout their life and their fitness is limited by their rate of egg maturation. To construct an exact model for synovigenic wasps would be very complicated and require information about the physiological mechanisms of egg production that we do not at present possess. However, the biological essence of this type of limitation can be captured using a disk equation: in the spirit of Charnov & Stephens (1988) and Hunter & Godfray (1995). Assume that the maximum number of eggs that can be laid per time unit is y. Then fitness is proportional to

$$W'(p',c',d') \propto \frac{1}{1+(1/y)\lambda(p'd'+(1-p')c')}[\lambda(p'd'g(d')v + (1-p')c'f(c'))] \quad (A5)$$

where the first term on the right-hand side of the equation is the rate at which eggs are deposited, and the second term (in square brackets) is the average fitness gain per host. Proceeding as before but defining a new index of host limitation $\phi_2 = y/\lambda$, we obtain the implicit relationships

$$\{p,c,d\} = \left\{ \frac{c+\phi_2}{c+d+2\phi_2}, \left(\frac{1}{c_L}+\frac{2(1-p)}{B+\phi_2} \right)^{-1}, \left(\frac{1}{d_L}+\frac{2p}{B+\phi_2} \right)^{-1} \right\}, \quad (A6)$$

where B, c_L and d_L are defined above. The results of this model are qualitatively very similar to the proovigenic model. Note, for example, that as $\phi_2 \to \infty$ then $\{p, c, d\} = \{0.5, c_L, d_L\}$.