

Seasonal variation in the sex allocation of a neotropical solitary bee

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We carried out a field study on the life history and sex allocation of the ground-nesting solitary bee *Diadasina distincta* (Hymenoptera: Anthophoridae). This species is multivoltine, undergoing five generations a year between February and September. The numerical sex ratio of this species was female biased overall (approximately 38% males) and showed a strong and consistent seasonal pattern. The numerical sex ratio was extremely female biased (approximately 20% males) from February until May, and then slightly male biased (approximately 60% males) from June until September. Females were 3.26 times the size of males, and so the overall investment ratio was female biased throughout the year. The overall female bias and seasonal variation in sex allocation is unlikely to be explained by models that invoke overlapping generations or competition between brothers for mates (local mate competition). We suggest that a possible explanation for the female bias in the early part of the season is local resource enhancement (LRE): nesting near larger numbers of sisters reduces parasitism. LRE is likely to decrease in importance in the later part of the season, when the biased numerical and investment ratios may be explained by models in which male and female offspring gain different fitness returns from resources invested. *Key words*: bees, *Diadasina distincta*, eusociality, local resource enhancement, sex ratio, variance. [*Behav Ecol* 10:401–408 (1999)]

The study of sex allocation has been one of the most successful areas of behavioral ecology (Charnov, 1982; Herre et al., 1999; Leigh et al., 1985). Frequency-dependent selection provides greater mating success for the rarer sex and so favors equal allocation of resources to sons and daughters (Fisher, 1930). If sons and daughters are equally costly to produce, then this equal investment also translates into equal numbers of males and females. This argument, however, assumes that the fitness returns from the production of sons and daughters are linear, or if not linear then identical (Frank, 1987b, 1990). Many different biological mechanisms break this assumption in which case-biased sex allocation is predicted. In these situations there is a rich experimental literature exploring the predictions of many theoretical models (Charnov, 1982; Godfray, 1994; Herre et al., 1997; Wrensch and Ebbert, 1993).

In this paper we are concerned with the predictions of sex allocation theory for solitary bees and wasps. Like other haplodiploid species, bees and wasps are able to control the sex of their offspring by whether or not they fertilize the egg: males develop from unfertilized eggs and females from fertilized eggs (Cook, 1993). Understanding the reasons for biased sex allocation in solitary bees and wasps is particularly important because they may have facilitated the evolution of eusociality (Grafen, 1986; Stubblefield and Charnov, 1986). A number of theories have been developed to predict and explain patterns of sex allocation in these species, and the available data are generally consistent with theoretical predictions (Brockman and Grafen, 1992; Cronin and Schwarz, 1997; Frank, 1987b, 1995; Helms, 1994; Rosenheim et al., 1996; Schwarz, 1988, 1994; Seger, 1983). However, support for these models is generally only qualitative, and detailed studies of individual species are required to test the assumptions as well

as the predictions of these models (Brockman and Grafen, 1992). To address this problem we have carried out a field study on the life history and sex allocation of *Diadasina distincta*, a solitary bee that nests in holes that females dig in the ground. Before describing our work we examine the various assumptions and predictions of the relevant theoretical models.

Werren and Charnov (1978; Seger, 1983) constructed a model to explain seasonal sex allocation biases in species with overlapping generations (partially bivoltine). The general prediction is that in any generation, selection favors an excess investment in the sex that has relatively greater overlap with future generations. For example, many species in the wasp family Sphecidae have two generations a year (Seger, 1983). Males and females diapause as late-instar larvae during the autumn, emerge in the spring, mate, and produce the offspring that will be the summer generation. The summer generation produce the offspring that diapause during the autumn. Some of the males that emerge in the spring may survive to mate females of the summer generation, as well as those of their own. In this case a male bias is favored in the offspring that diapause over winter, and a female bias in the other generation. These models provide a possible explanation for the seasonal sex allocation patterns that have been observed in partially bivoltine wasp and bee species (Brockman and Grafen, 1992; Seger, 1983). Seasonal sex allocation patterns may also evolve in response to seasonal perturbations (variation in recruitment or mortality rates) of the population (Werren and Charnov, 1978; Werren and Taylor, 1984; West and Godfray, 1997).

Frank (1987b, 1995; see also Charnov et al., 1981; Trivers and Willard, 1973) considered the situation when male and female offspring gain different fitness returns from the resources invested for offspring provisioning, such as hosts or pollen. For example, suppose that females gain more from an increase in size than males. In this case theory predicts (1) more resources should be provided to females, who will subsequently be larger; (2) the investment sex ratio (defined as

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Received 21 July 1998; revised 13 November 1998; accepted 21 December 1998.

the proportion of resources allocated to males) should be female biased; (3) the numerical sex ratio (defined as the proportion of males) should be male biased; and (4) seasonal variation in either the distribution of resources or the relationships between fitness and size should lead to a change in the relative size of the sexes, the overall investment sex ratio, and the numerical sex ratio.

More recently, Rosenheim et al. (1996) have extended this type of model to the case when egg supply may also limit reproduction. In this case the factor limiting a female's reproduction may vary across a continuum from purely egg limited to purely resource limited, with intermediate states where a female is partially limited by both. There is observational and experimental evidence that female parasitoid wasps facultatively adjust their behavior in response to such variation (Hunter and Godfray, 1995; West et al., 1999). Rosenheim et al.'s model makes two additional predictions. As the availability of resources for provisioning increases and females become more egg limited they should increase provision masses per offspring and produce a less male biased or even an equal numerical sex ratio and a more female biased investment sex ratio.

Another factor that has been suggested to lead to female-biased sex allocation in solitary bee species is local resource enhancement (LRE) (Schwarz, 1988). Local resource enhancement can occur when the presence of individuals of one sex increases the fitness of other related individuals (Greeff, 1999; Seger and Charnov, 1988; Taylor, 1981; Toro, 1982). This has been shown to potentially be important in explaining the female-biased sex allocation of some bee species where related females cooperate to build and use nests (Cronin and Schwarz, 1997; Schwarz, 1988, 1994; Stark, 1992). An important point is that LRE between siblings can result in two outcomes, depending on whose fitness is increased (Greeff, 1999; Seger and Charnov, 1988; Toro, 1982). If both sexes benefit, then an overall bias in sex allocation is favored, with all females investing in the same way. However, if only the sex that provides the benefit gains a fitness increase, then, although the overall sex allocation may still be biased, there is no single investment ratio that all females should produce. Instead, some individuals are predicted to produce purely males and the remainder to produce purely females, leading to greater than binomial (random) variance in the numerical offspring sex ratios.

In this paper we examine the neotropical solitary bee, *D. distincta*. This species has several overlapping generations per year (Martins and Antonini, 1994). Consequently, any of the above sex ratio models could theoretically be applicable, and so it is an excellent species on which to carry out a detailed study. Our first aim was to document the numerical and overall investment sex ratio of the species and any seasonal variation. Our second aim was to test some of the assumptions and predictions of the various theoretical models described above. We determined the population dynamics of the species so that we could evaluate the importance of models which invoke overlapping generations. To examine the role of models where males and females gain different fitness returns from resources, we determined (1) the availability of the resource (pollen) used to provision offspring; (2) whether there was any seasonal variation in the number of nests produced; (3) the amount of pollen used to provision nests containing males and females, and (4) the relative size of males and females. Finally, we assessed the importance of LRE by documenting the prevalence of parasitism and the variance in numerical offspring sex ratios produced by individual females.

Background biology

D. distincta is a neotropical ground-nesting bee (Martins and Antonini, 1994). We have studied populations in a 3×50 m

study site at the Ecological Station of the Universidade Federal de Minas Gerais (EE-UFGM) in Belo Horizonte, Minas Gerais, Brazil (see Martins and Antonini, 1994). The adult bees emerge at the end of the rainy season during February, and nesting activities occur until October, through the drier and colder months of the year. Females nest in aggregations, with the size of aggregations varying seasonally (Martins and Figueira, 1992). In the beginning of the reproductive season aggregations are small (20–50 nests), but they then grow in size until August (50–2000 nests), after which they decrease in size until the end of the reproductive season. Each nest is a burrow (single cell) in the ground approximately 4 cm deep and 1 cm in diameter. A single female constructs each nest, and old nests are not reused. Females collect pollen, predominantly (>90%) from *Ludwigia laruotheana* (Onagraceae; Martins and Borges, in press), and mold it into a ball in at the bottom of each nest. They then lay a solitary egg under this ball and close the nest entrance with moist soil. Excavating, provisioning, and closing a nest takes up to 5 days. The egg–adult development time observed in the field is approximately 27 days. Adult females sleep in nests, and males sleep in the surrounding vegetation on which they also rest between patrolling periods. On sunny days the males search for mates in aggregations. Males strongly compete for females and pursue both newly emerged females and females provisioning nests. Females mate repeatedly throughout their lives.

METHODS

Population dynamics and parasitism

We estimated the bee population size (number of male and female bees in our study site) every 15 days from March to September during 1994 and 1995. The distance between aggregations in our study site averaged approximately 1.5 m. We used sweep nets to capture all the individuals flying over nest aggregations at the peak of daily activity. Sweeping was done over relatively large aggregations (30–50 nests at the beginning of the reproductive season): 6 in 1994 and 4 in 1995. The captured individuals were marked with a fast-drying acrylic paint and released. Twenty four hours later we captured all flying individuals using the same technique. We then estimated the population size by the Petersen method (Bailey, 1952). We also estimated pollen availability every 15 days over the same periods by counting the number of receptive flowers on 20 marked individuals of the plant *L. laruotheana*. Flowers of *L. laruotheana* are receptive for 1 day. This species is the main (>90%) source of pollen for female nests (Martins and Borges, in press).

We examined the demography of the population in further detail by marking and following individual bees and their offspring. Females from the first generation in 1994 and 1995 were marked as above and followed. Plastic cups were placed over any nests that they built and observed daily. Emerging adults were sexed and females marked. Emerging parasites were recorded. Cups were placed over all nests produced by the marked females. This was repeated with the subsequent generations until the end of the reproductive season. By September, the population was composed entirely of dormant larvae (Martins and Antonini, 1994), and we observed the nests containing these larvae until the emergence of adults the following year. Ten days after the emergence of the last adult in a generation we excavated the remaining nests. In these cases the nest contents were generally moldy pollen balls or, rarely, dead adults. We estimated the longevity of females by recording the number of days that marked individuals from the first generation of 1994 were observed at the nest aggregation after their emergence.

Sex allocation

We estimated the numerical sex ratio by recording the sex of adults caught in emergence traps placed over six nest aggregations. These six aggregations were sampled every month during 1994 and 1995. The nests examined covered a range of sizes, varying between 30 and 50 nests at the beginning of the reproductive season. These traps caught all the bees that emerged and so could not be biased by different behaviors of the two sexes.

We also recorded the numerical offspring sex ratios produced by individual females. This was done by recording the offspring sex ratios produced by the females that we had individually marked. Plastic cups were placed over all the nests constructed by marked females, and the sex of the emerging offspring recorded. An important caveat with this data is that it was only collected on individuals that were in small aggregations (<50 nests throughout the reproductive season). Marked individuals could not be followed in large aggregations. This will have biased our results if the sex ratio differed with aggregation size.

Sexual dimorphism

We measured the width of the thorax in males and females. This measurement was taken as the maximum distance between the external margins of the tegulae. We cubed the thorax width, as this has been shown to be highly correlated with dry weight (Silveira et al., 1993). The females measured were collected during April and August for both 1995 and 1996. The males measured were collected during April 1995, April 1996, and August 1996. We used these measurements in conjunction with the observed numerical sex ratios to estimate the overall investment sex ratio (see Boomsma, 1989, for why this may underestimate the overall allocation to females). We also measured the length and diameter of cells from which males and females had emerged. These cells had been brought into the laboratory during May 1993.

Statistical analysis

All analyses were carried out with the GLIM statistical package (Crawley, 1993). Proportion data such as sex ratio (or proportion parasitized) usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems, we analyzed all proportion data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function. The number of males in a sample was used as the response variable and the total number of males and females as the binomial denominator. Initially, a full model was fitted to the data, including all explanatory variables and their interactions. Terms were then removed from the full model by stepwise deletion (Crawley, 1993). Whether the removal of a term caused a significant increase in deviance was assessed with a chi-square test.

The variance in the numerical offspring sex ratios produced by individuals were analyzed with the two methods used and described in detail by West and Herre (1998). The rationale behind both methods was to compare the variance observed in the sex ratio data with that expected given a binomial (random) distribution. One of the methods is based on least squares regression (GV; Green et al., 1982; West and Herre, 1998) and the other on maximum likelihood (HF; West and Herre, 1998). The values of GV and HF represent the observed variance divided by that expected given a binomial distribution. Values of GV or HF < 1 indicate less than binomial variance, and values of GV or HF > 1 show greater than binomial variance.

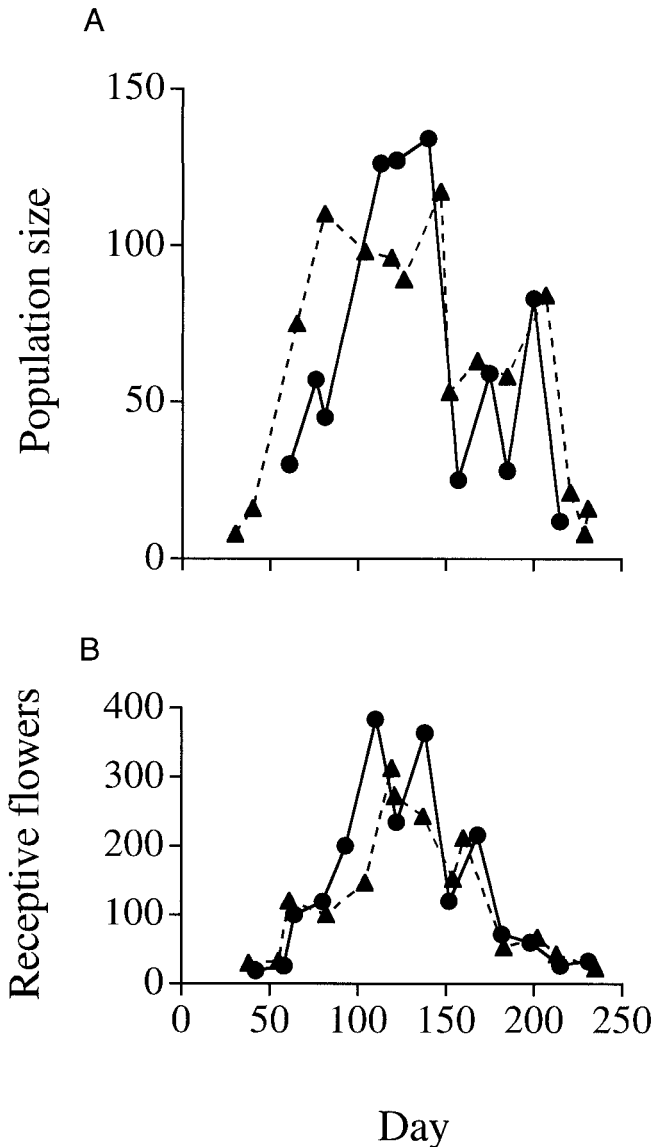


Figure 1
Seasonal variation in (a) the population size of *D. distincta* and (b) the number of receptive flowers of *L. laruotteana* for 1994 (circles and solid line) and 1995 (triangles and dashed line). Day 1 represents February 1.

RESULTS

Population dynamics and parasitism

Population size showed a strong seasonal pattern, with a similar pattern observed in 1994 and 1995. The number of bees increased until June, after which time it declined (Figure 1a). The population dynamics of the bee strongly followed that of its host plant, *L. laruotteana*. The number of receptive flowers on 20 marked plants increased until May (1994) or June (1995), after which time the number declined (Figure 1b).

The number of generations in a year was determined by marking and following individual bees and their offspring. There were five generations in both 1994 and 1995. All of the offspring of the fifth generation entered diapause. No offspring from earlier generations entered diapause. The periods between which the first and last female emerged each generation are shown in Figure 2. There was some overlap in emergence time between the first and second generations

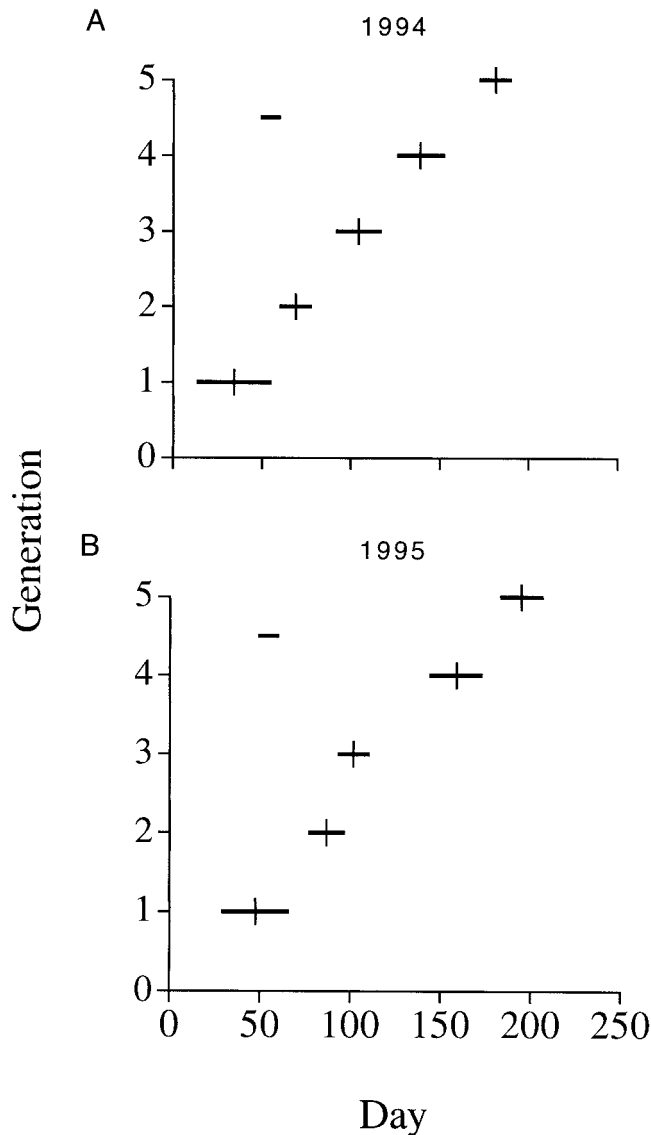


Figure 2
The emergence dates of the generations of *D. distincta* during (a) 1994 and (b) 1995. Shown are means \pm SDs of the emergence dates of bees from each generation. Day 1 represents February 1. Also shown is a bar representing the average longevity (9.0 days) of females.

(both years) and between the second and third generations (1995 only).

The marked females produced between two and five nests during their lifetime. The average number of nests produced by a female was 2.90 (95% CI, 0.09, $n = 270$), and this did not differ significantly between generations ($F_{4,265} = 0.91$, ns). The proportion of a female's nests that were parasitized in 1994 varied significantly between generations ($\chi^2_{(4)} = 15.19$, $p < .005$, Table 1), and showed no significant relationship with the number of nests that the female produced ($\chi^2_{(1)} = 0.99$, $p > .1$). Within generations, neither male nor female offspring emerged consistently before the other. The longevity of the marked females in the first generation of 1994 ranged from 6 to 12 days and averaged 9.00 (95% CI, 1.20).

Sex allocation

In both 1995 and 1996 the numerical sex ratio of emerging bees was strongly female biased (approximately 20% males)

Table 1
The proportion of nests that were parasitized in the different generations during 1994

Generation	No. of females followed	Proportion of nests parasitized (95% CI)
1	13	0.25 (0.13–0.42)
2	23	0.12 (0.06–0.23)
3	45	0.06 (0.03–0.12)
4	69	0.04 (0.02–0.09)
5	120	0.07 (0.05–0.11)

Data are from the nests constructed by the females that were marked and followed. The 95% confidence intervals were calculated with logit transformations.

from March until June (corresponding to the first three or four generations), and then slightly male biased (approximately 60% males) from July until September (corresponding to the last one or two generations; Table 2 and Figure 3a). Summing over the relevant months, these numerical sex ratios were significantly different from 50% males for both March until June (1995: $\chi^2_{(1)} = 148.70$, $p < .001$; 1996: $\chi^2_{(1)} = 121.20$, $p < .001$) and July until September (1995: $\chi^2_{(1)} = 7.71$, $p < .01$; 1996: $\chi^2_{(1)} = 17.90$, $p < .001$). The numerical sex ratio differed significantly between months ($\chi^2_{(1)} = 220.90$, $p < .0001$), but not between years ($\chi^2_{(1)} = 1.88$, $p > .1$). Summing over both years, the overall numerical sex ratio was significantly female biased ($\chi^2_{(1)} = 80.50$, $p < .0001$; 38% males).

The pollen used for stocking nests, or the time required to locate and collect the pollen, may be factors limiting the sex allocation of females. Two possible indices of pollen availability in a month are the number of receptive flowers and the number of receptive flowers divided by the average number of foraging female bees (the estimated population size). The numerical sex ratio of emerging bees showed no correlation with either the number of receptive flowers ($\chi^2_{(1)} = 0.12$, $p >$

Table 2
The numerical and investment offspring sex ratios of bees caught in emergence traps placed over nest aggregations during 1994 and 1995

Year/month	Numerical sex ratio (n^a)	Investment sex ratio ^b
1994		
March	0.15 (100)	0.05
April	0.20 (100)	0.07
May	0.23 (100)	0.08
June	0.24 (100)	0.09
July	0.59 (100)	0.31
August	0.60 (100)	0.32
September	0.55 (100)	0.27
1995		
March	0.20 (115)	0.09
April	0.20 (75)	0.09
May	0.25 (88)	0.11
June	0.25 (103)	0.12
July	0.57 (124)	0.51
August	0.73 (85)	0.42
September	0.60 (60)	0.36

^a Number of individuals sexed to estimate the ratio.

^b The investment sex ratio ratios were estimated for 1994 and 1995 using the average measure of male and female size from 1995 and 1996, and the measures from 1995 only, respectively.

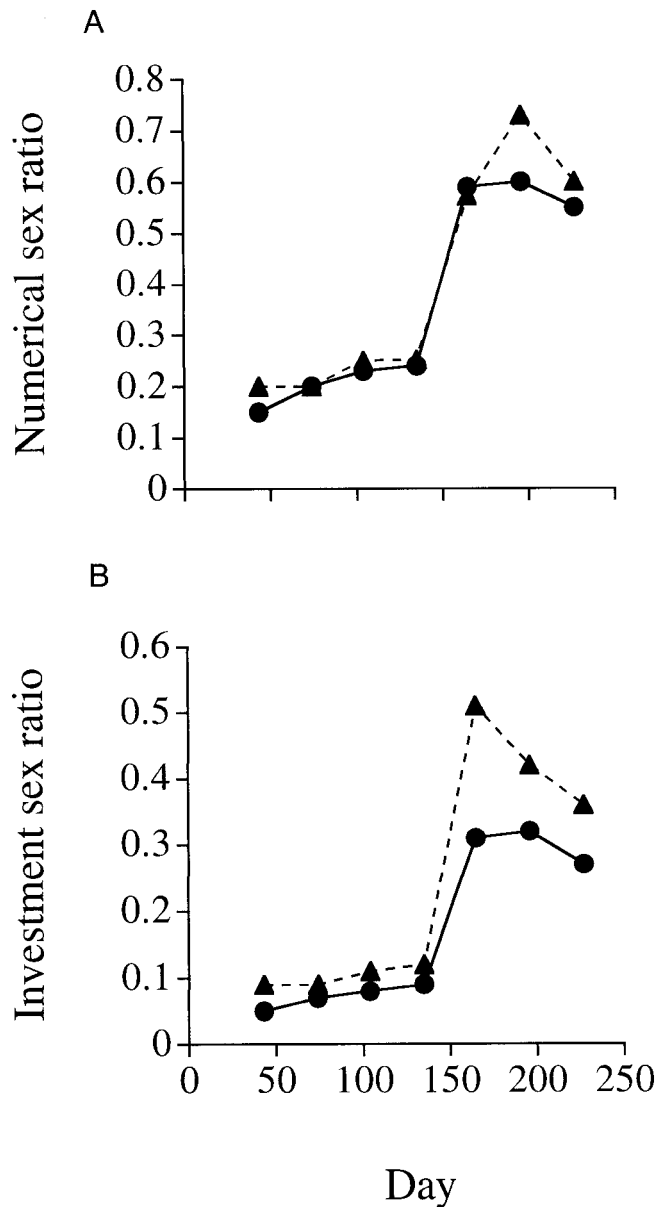


Figure 3
The (a) numerical and (b) investment offspring sex ratios of bees caught in emergence traps placed over nest aggregations during 1994 (circles and solid line) and 1995 (triangles and dashed line). Day 1 represents February 1.

.1) or the number of receptive flowers divided by the average number of foraging female bees ($\chi^2_{(1)} = 2.52, p > .1$). There is a large discontinuity in the numerical sex ratios between June and July, so we also tested for correlations separately in these two parts of the year. In both cases there was no significant correlation with either the number of receptive flowers (March–June: $\chi^2_{(1)} = 3.73, p > .05$; July–September: $\chi^2_{(1)} = 0.35, p > .1$) or the number of receptive flowers divided by the average number of foraging female bees (March–June: $\chi^2_{(1)} = 3.81, p > .05$; July–September: $\chi^2_{(1)} = 0.17, p > .1$), although it should be noted that the same close-to-significance positive correlation from March to June was observed in both years.

We also collected data on the numerical offspring sex ratios produced by individual marked females during 1994 (Table

Table 3
The numerical offspring sex ratios, and their variation, produced by individual marked females during 1994

Generation	n^a	Numerical sex ratio (95% CI) ^b	GV ^c	HF ^c
1	11	0.15 (0.06–0.34)	0.57	0.60
2	21	0.17 (0.09–0.29)	0.54	0.58
3	43	0.27 (0.20–0.36)	0.79	0.98
4	67	0.25 (0.19–0.32)	0.79	0.76
5	118	0.40 (0.35–0.46)	0.69*	0.79

^a Number of individuals whose offspring were recorded in each generation.

^b The 95% confidence intervals were calculated with logit transformations.

^c GV and HF are two estimates of the degree of variance in the numerical sex ratio, values <1 representing less than binomial variance.

* $p < .001$.

3). The numerical sex ratio was highly female biased (approximately 15% males) in the first two generations, less female biased (approximately 25% males) in the third and fourth generations, and slightly female biased (approximately 40% males) in the fifth generation. This difference between the generations was significant ($\chi^2_{(4)} = 24.80, p < .001$), and was significantly positively correlated with the number of nests that a female produced ($\chi^2_{(1)} = 28.32, p < .001$). The slope of this relationship (the interaction term) did not differ significantly between generations ($\chi^2_{(4)} = 3.26, p > .1$). The numerical sex ratio was significantly different from the expected 50% males in all five generations. Overall, the numerical offspring sex ratio produced by the marked females was significantly more female biased than that caught in emergence traps placed over the aggregations (1995: $\chi^2_{(1)} = 4.52, p < .05$; 1996: $\chi^2_{(1)} = 9.49, p < .005$).

Overall, the numerical sex ratios produced by individuals showed significantly less variation than that expected with a binomial distribution (GV = 0.82, $p < .001$; HF = 0.79). Examining the numerical sex ratios from each generation separately also showed less than binomial variation in each generation, although this difference was only significant for generation five (Table 3). However, sample sizes and thus the power of the test were lower for the other generations.

Size dimorphism

Females were significantly larger than males ($F_{1,74} = 161.74, p < .01$, Table 4). Female size did not differ significantly be-

Table 4
The size of male and female bees

Sex	Year	Month	n	Average size, mm (95% CI)
Female	1995	April	14	74.43 (16.11)
		August	12	66.00 (7.27)
	1996	April	10	73.32 (12.31)
		August	10	68.64 (15.94)
Male	1995	April	10	26.93 (2.98)
	1996	April	10	18.52 (2.35)
		August	10	19.86 (3.61)

Bee size was measured in 2 years at the beginning (April) and end (August) of the reproductive season.

tween the beginning (April) and end (August) of the reproductive season ($F_{1,44} = 1.25$, ns), or between 1995 and 1996 ($F_{1,43} = 0.01$, ns). The interaction between these two factors (stage of season and year) was also not significant ($F_{1,42} = 0.09$, ns). Male size did not differ significantly between the beginning and end of the reproductive season ($F_{1,27} = 0.52$, ns). Males were significantly smaller in 1996 than in 1995 ($F_{1,28} = 161.71$, $p < .01$). Females were 2.62 and 3.70 times the size of males for 1995 and 1996, respectively; these estimates were used to calculate the overall investment sex ratios which are given in Table 2.

Females developed in significantly larger nests (cells) than males (length: $F_{1,38} = 45.57$, $p < .01$; diameter: $F_{1,38} = 40.45$, $p < .01$). The average length and diameter of female nests were 1.14 mm (95% CI, 0.03, $n = 25$) and 0.85 mm (95% CI, 0.03, $n = 25$), respectively. The average length and diameter of male nests were 0.97 mm (95% CI, 0.04, $n = 15$) and 0.71 mm (95% CI, 0.04, $n = 25$), respectively.

DISCUSSION

We have collected life-history and sex ratio data on the neotropical solitary bee, *D. distincta*. This species is multivoltine, undergoing five generations a year between March and September (Figure 2). The numerical sex ratio of emerging bees in this species showed a strong and consistent seasonal pattern (Figure 3a), and this is the first time this pattern has been observed in a neotropical bee species. The numerical overall sex ratio was extremely female biased (approximately 20% males) from February until May (corresponding to the first three to four generations), and then slightly male biased (approximately 60% males) from June until September (corresponding to the last one or two generations). Summing over the year, the overall numerical sex ratio was significantly female biased (38% males). Estimates from 1995 and 1996 suggest that females were, on average, 3.25 times larger than males, and so the overall investment ratio was female biased throughout the year (Figure 3b).

Variable sex allocation may evolve in response to a number of factors in species with overlapping generations, such as variation between the generations in diapause, population recruitment, or mortality rates (Seger, 1983; Werren and Charnov, 1978; Werren and Taylor, 1984; West and Godfray, 1997). The general prediction of these models is that selection favors an excess of the sex that will experience lower reproductive competition (West and Godfray, 1997). Variable diapause between the generations is unlikely to be important in *D. distincta* because only individuals of the fifth generation diapause, and all of these individuals emerge in the first generation the following year. Adults do not survive over winter. Likewise, variation in either population recruitment or mortality rates is unlikely to be important because the overlap between generations is not of a form predicted to lead to sex allocation biases: female longevity (approximately 9 days) is considerably less than the egg-to-adult development time (approximately 27 days), and so individuals are unlikely to compete with their own offspring. We have not measured male longevity, but even if it were enormously greater, it could not explain the female bias in earlier generations—it would actually favor a bias in the opposite direction toward males.

Frank (1987b, 1990, 1995) predicted the consequences of differing relationships between the resources invested for offspring provisioning and fitness differ between the sexes. In *D. distincta*, more resources were invested in female offspring, who were consequently larger. This suggests that females gain more from increased resources than males, a pattern which is believed to hold across many hymenopteran species (Charnov et al., 1981; Godfray, 1994). In this case Frank's models pre-

dict that the overall numerical sex ratio will be male biased, and the investment sex ratio female biased. Frank's models therefore predict the observed patterns in both the numerical and investment sex ratios during the latter part of the season, corresponding to the last one or two generations. However, these models cannot predict the extreme female bias in both the numerical and investment sex ratio during the earlier part of the season.

Rosenheim et al. (1996) extended the type of model considered by Frank to include egg limitation. Rosenheim et al. predict that as the total resources available decreases, fewer resources will be provided for each offspring and that, when males are the smaller sex, the numerical sex ratio should become more male biased. However, we found no significant correlation between the numerical sex ratio and possible correlates of the amount of resources (pollen) available for provisioning offspring: the number of receptive flowers and the number of receptive flowers per female bee.

We have concentrated on discussing the overall sex allocation of emerging bees (Figure 3). However, we also examined the numerical offspring sex ratios produced by individual females (Table 3). An important caveat with this data is that it was only collected on individuals that were in small aggregations; marked individuals could not be followed in large aggregations. This would have biased our results if the sex ratio differed with aggregation size. The offspring sex ratios produced by these females became less female biased in the later generations but did not become male biased like the data collected by emergence traps over the aggregations. The data collected on individual sex ratios showed that the largest sex ratio shift was between the last two generations of the year. This is consistent with the male bias in the emergence-trap numerical sex ratios during the latter part of the season being due primarily to the fifth generation.

The numerical offspring sex ratio produced by individual females was significantly positively correlated with the number of nests that they produced. This correlation would be predicted by Frank's (1987b, 1995) models if females producing more offspring also provided fewer resources to each individual offspring. Future work could test this possibility.

A commonly mentioned explanation for female-biased sex ratios in hymenopteran species is competition for mates between brothers and inbreeding in subdivided populations [termed local mate competition (LMC); Hamilton, 1967]. The biology of the species makes LMC seem unlikely: each nest contains only a single egg, males search for mates in aggregations, and females mate repeatedly throughout their lives. A role for LMC seems particularly unlikely, as either extreme competition between brothers or large (>50%) numbers of matings between siblings would be required to explain the extreme female bias observed. In addition, we observed a positive correlation between sex ratio and brood size, which is the opposite direction to that predicted and observed under conditions of LMC (Flanagan et al., 1998; Griffiths and Godfray, 1988; Stubblefield and Seger, 1990; Werren, 1980; West et al., 1997). More generally, there is no evidence for LMC in solitary wasps and bees (Frank, 1995).

We suggest that a possible explanation for the extreme female bias in both the numerical and investment sex ratios during the early generations is LRE. Parasitism would provide the driving force given the following scenario. Parasitism is lower in large aggregations (Antonini Y, Martins RP, in preparation), and so the fitness of females is likely to be greater in larger aggregations. Female-biased offspring sex ratios have at least two consequences that would lead to fitness benefits: (1) females stay in the same area and nest near their sisters (Antonini Y, Martins RP, in preparation), and so having more sisters leads to being in a larger aggregation and (2) because

the population is growing in the first generations of the year (Figure 1), having more sisters would lead to future generations (children, grandchildren, etc.) nesting in exponentially larger aggregations (see also Frank, 1987a).

A testable hypothesis arising from this scenario is that the female bias in both the numerical and investment sex ratios would be greater in smaller aggregations. Interestingly, the numerical offspring sex ratios produced by the individual marked females, who were in relatively small aggregations, were significantly more female biased than the numerical sex ratio of bees caught in emergence traps placed over aggregations. However, further work on how males disperse is required to back up this prediction. For example, if males dispersed to other aggregations, then females nesting in small aggregations might be favored to produce males, who could go to large aggregations to mate with females whose offspring would suffer from relatively low parasitism. Theoretical work would be required to understand the relative consequences of these different factors. Predictions may also be complicated by the fact that the optimal strategies for individuals to pursue (e.g., sex allocation and dispersal) and the population dynamics will both have consequences for and depend upon each other.

To fully explain our data, this hypothesis would require that LRE becomes less important in the later generations, especially the fifth. Local resource enhancement would be expected to be most important (and therefore predict a greater female bias) in the early part of the season: early in the year aggregations are small (20–50 nests) and parasitism is high (Table 1), whereas later in the year aggregations are large (50–2000 nests) and parasitism is low (Table 1). Future work will be required to determine whether this is able to explain the extreme jump, in both the emergence trap and individual numerical sex ratio data, between the last two generations of the year.

Local resource enhancement theory predicts that if only females benefit from having extra sisters, then some individuals should produce only daughters, and the rest of the population only sons (Greeff, unpublished manuscript; Seger and Charnov, 1988; Toro, 1982). This would lead to an overdispersed pattern of individual sex allocation and much greater variance than that expected given a binomial distribution. However, we observed the opposite pattern, significantly less than binomial variation. This would be predicted under LRE if both sexes gained a fitness advantage from extra sisters. A possible explanation for this is that the benefit through an increased growth rate and larger aggregations in future aggregations would benefit both sexes. Another possibility is that some important factor remains to be documented. However, it should also be pointed out that our analyses were based on the numerical sex ratios of individual females in relatively small aggregations. Thus we do not know the sex ratio variance among females in larger aggregations (where females may produce more sons), nor in the total population.

The observed positive correlation between numerical sex ratio and brood size was in the same direction as that previously noted in allodapine bee species where the female-biased sex allocation is most likely due to LRE (Cronin and Schwarz, 1997; Schwarz, 1994). The theoretical basis of such a correlation is unclear (Greeff, 1999). An important difference with our study is that the allodapine species studied are primitively (facultatively) eusocial, with related females cooperating to build, use, and defend nests. Related females of *D. distincta* merely nest near each other and do not actively cooperate. Nonetheless, the association between related females nesting near each other and female-biased sex ratios could certainly help facilitate the evolution of eusociality and could represent a primitive step on the way toward cooperation.

To conclude, our data on *D. distincta* support the importance of some areas of sex ratio theory and allow others to be rejected. Both female offspring gaining relatively more than males from increased resources and LRE appear to be strong possibilities, and we suggest that their relative importance varies seasonally. In contrast, any effect due to overlapping generations or LMC seems unlikely. Further work is required and should be centered around the models that our current study suggest are likely to be important. Empirical studies on this and related species are urgently required to determine the relationship between size and fitness for both sexes under field conditions (e.g., Visser, 1994; West et al., 1996), examine the relationships between resources invested and adult body size for the two sexes, and test how the fitness of an individual female and the offspring sex ratio is affected by the size of an aggregation. Theoretical work is required to provide sex allocation models that make quantitative predictions from LRE. More generally, this is also true for a range of insect and vertebrate examples (e.g., Komdeur et al., 1997; Lambin, 1994; Schwarz, 1988; Stark, 1992) where within- and/or cross-generational interactions can lead to LRE.

We thank James Cook, Jaco Greeff, Ashleigh Griffin, Jack Werren, and Doug Yanega for useful discussion and comments on the manuscript; Arturo Roig-Alsina, N. Evenhuis, and Z. Boucek for identification of insects; the Biotechnology and Biological Sciences Research Council, Brazilian National Research Council, Minas Gerais State Research Funding Agency, and the U.S. Fish and Wildlife Service for funding. This is a contribution of the program in ecology and wildlife management of the Federal University of Minas Gerais.

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