

## ORIGINAL ARTICLE

Lesley Hughes · Belinda Siew-Woon Chang  
Diane Wagner · Naomi E. Pierce

## Effects of mating history on ejaculate size, fecundity, longevity, and copulation duration in the ant-tended lycaenid butterfly, *Jalmenus evagoras*

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**Abstract** The mating system of the Australian lycaenid butterfly, *Jalmenus evagoras*, is highly unusual compared to most other Lepidoptera. Characteristics of this system, which has been termed an ‘explosive mating strategy,’ include the formation of an intensely competitive mating aggregation of males, a highly male biased operational sex ratio, a lack of discrimination and mate choice by both sexes, a high variance in male mating success, and female monogamy. In this study, we tested the hypothesis that multiple mating by males imposes physiological costs resulting in smaller spermatophores, and that this results in a fitness cost to females. We found that male *J. evagoras* transferred only 2.2% of their eclosion weight during their first mating, consistent with the hypothesis that males of monandrous species produce a relatively small investment. The wet weight of the ejaculate declined by an average of 27% at the second mating and the dry weight by 29%, and an intermating interval of 5–9 days was needed for the ejaculate to return to the size at the first mating, regardless of male size or age. Wet ejaculate mass increased proportionally with male size, though dry mass was proportionally large-

er in smaller males. Ejaculate mass tended to increase with male age at both first and second matings. Female characteristics, in general, did not affect ejaculate mass, although the wet weight of the ejaculate was positively associated with female weight at the second mating. Copulation duration increased from 2.4 h to approximately 3 h at the second mating, and to over 4 h at the third and fourth matings. Fecundity was positively correlated with female size but not with mating history, copulation duration, or any other characteristics measured for either males or females. Female longevity declined significantly as the number of times the male partner had previously mated increased. We conclude that despite the small male investment in ejaculate, the costs of multiple mating may nonetheless be significant, as indicated by the reduction in ejaculate mass, an increase in copulation duration, and reduction in female lifespan with increasing mating number.

**Key words** Copulation duration · Explosive mating system · Ejaculate size · Lepidoptera · Mating history

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L. Hughes (✉)<sup>1</sup> · N.E. Pierce  
Museum of Comparative Zoology, Harvard University  
Cambridge, MA 02138, USA

B. Siew-Woon Chang<sup>2</sup> · D. Wagner<sup>3</sup>  
Department of Ecology and Evolutionary Biology  
Princeton University, Princeton, NJ 08544, USA

*Present addresses:*

<sup>1</sup>Department of Biological Sciences  
Macquarie University, North Ryde, NSW 2109, Australia  
e-mail: lhughes@rna.bio.mq.edu.au  
Tel.: +61-2-98508195  
Fax: +61-2-98508245

<sup>2</sup>Department of Organismic and Evolutionary Biology  
Harvard University, 22 Divinity Avenue, Cambridge  
MA 02138, USA

<sup>3</sup>Department of Biological Sciences  
University of Nevada, Las Vegas, NV 89154, USA

### Introduction

Mating behavior in the Lepidoptera has been increasingly used in recent years to examine how ecological circumstances constrain or promote sexual selection and the evolution of different mating systems. In many butterfly species, males do not defend either mates or a territory, conforming to a category of mating system known as *scramble competition polygyny* (Emlen and Oring 1977). Within this category, two alternate types of mating behavior have been distinguished, based on the availability of females. When females are spread out in time and/or space, a *prolonged searching polygyny* may be favored, in which males fly about broadly in search of receptive females in an effort to outrace their competitors (Thornhill and Alcock 1983). Under these circumstances, males may be expected to indulge in complex courtship behavior to stimulate unreceptive females and both

sexes may be expected to both exhibit some degree of mate choice, and to mate several times over their lifetime (Thornhill and Alcock 1983; Odendaal et al. 1985). The majority of butterflies display this type of mating system (Scott 1974), and while behavior in courtship varies among species (reviewed by Scott 1972; Silberglied 1977; Rutowski 1984a, 1991), most differences are modest variations on the same basic plan. After locating a female, a male typically approaches and courts her by either buffeting her with his wings or with a simple display. The male then attempts a copulation and if the female is receptive, copulation ensues (Silberglied 1977).

When receptive females are highly aggregated in either time or space, an *explosive breeding* strategy, in which males congregate to compete for females, may be favored (Thornhill and Alcock 1983; Rutowski 1984a). Odendaal et al. (1985) have suggested that a suite of traits might be expected in butterfly species that have adopted this breeding strategy, including a highly male biased operational sex ratio, poor discrimination among mates, and strategies by males that ensure quick access to fertilizable females. They also suggested that females of explosive breeders may be expected to be mated immediately upon eclosion or upon entering male aggregations, to have less need to discriminate among mates because winners in male-male competition automatically have higher mating success, and to have a lower tendency than prolonged searchers to mate more than once. Odendaal et al. (1985) further suggested that the position of a butterfly species along the behavioral continuum from prolonged searching to explosive breeding is determined to a large extent by the ecological conditions facing the larvae. Specifically, they hypothesized that the presence of host plants in appropriate condition determines the duration of availability of fertilizable females, and this in turn determines male mating strategies. Rutowski et al. (1988) further suggested that the spatial distribution of pupation sites, and thus of freshly eclosed females, have a profound effect on mating strategy.

Explosive breeding systems have been described for relatively few non-territorial butterflies. Several species in the genus *Euphydryas* display many of the characteristics described above, including the brief availability of fertilizable females, a male-biased operational sex ratio, and poor discrimination by males between adults of the different sexes (Odendaal et al. 1985). Many species of *Heliconius* have pupal mating, in which males compete with each other for access to female pupae and actually mate before the female has fully eclosed (Deinert et al. 1994). The mating frenzy of monarch butterflies, *Danaus plexippus*, that occurs just before they disperse from their winter aggregations, may also qualify (Rutowski 1984a).

The subject of this study, the Australian lycaenid *Jalmenus evagoras*, appears to represent an extreme manifestation of the explosive breeding strategy as characterized above, and provides support for the idea that both larval ecology and the distribution of pupation sites are important determinants of adult mating strategy. *J. ev-*

*agoras* larvae are obligately dependent on ants in the genus *Iridomyrmex* for protection against predators and parasitoids (Pierce et al. 1987). Females use the presence of ants as a cue for oviposition (Pierce and Elgar 1985), and frequently oviposit on the same host tree from which they eclosed as pupae. *J. evagoras* populations are very patchily distributed, confined to locations where the appropriate combination of *Acacia* host plants and suitable ant species co-occur (Smiley et al. 1988). Males eclose several days before females and start searching for mates by visiting pupae on various trees. Pupae are easily visible because they tend to aggregate and are tended by many ants (Elgar and Pierce 1988). The males hover near each pupa in turn, tapping it with their antennae, possibly to determine its age and its nearness to eclosion. About 12 h before emergence, pupae secrete substances that attract males and upon eclosion become surrounded by a 'mating ball' consisting of as many as 20 male butterflies. The males scramble and butt each other in their attempts to ward others off and copulate with the newly emerging butterfly. Despite the fact that female pupae are often larger, males are apparently unable to distinguish the sex of the emerging butterfly and are just as likely to attempt to copulate with newly eclosing males as females (Elgar and Pierce 1988). Once the wings of the females have hardened, they vigorously reject further attempts at copulation by males; field observations of marked butterflies suggest they do not mate again (N.E. Pierce, personal observation). There is high variability in male lifetime breeding success, with the majority of matings being accomplished by a small number of individual males (Elgar and Pierce 1988). Relatively larger and older males are more successful at competing for mates than smaller and younger males. Females appear to live only a short time in the field (~3 days, compared to 7 for males), and the operational sex ratio is therefore highly male biased (Elgar and Pierce 1988).

The general aim of this study was to measure some of the characteristics and costs of this unusual breeding system for comparison with other species that exhibit more typical lepidopteran reproductive behavior. Specifically, we tested the hypotheses that multiple mating by males imposes a physiological cost on males in terms of ejaculate mass, copulation duration, and refractory interval, and that this in turn may affect female fitness in terms of fecundity and longevity. These costs may be particularly important in an explosive breeding system where most matings are accomplished by just a few males during intense physical competition. We addressed the following questions.

First, what proportion of the males' body weight is transferred during mating, compared with other butterfly species? Relative ejaculate mass in butterflies has generally been found to increase with degree of polyandry (Svärd and Wiklund 1989; Karlsson 1995). We predicted, therefore, that the monandrous *J. evagoras* would produce a relative small spermatophore.

Second, given that most matings in *J. evagoras* are accomplished by just a few males, what are the costs of

multiple mating? In one field study, Elgar and Pierce (1988) found that the most successful male *J. evagoras* mated seven times and that 57% of males failed to mate at all. Mating history has been shown in several butterfly species to have significant effects on both the quantity of material transferred at mating, and the time spent in copula (e.g., Boggs and Gilbert 1979; Rutowski and Gilchrist 1986; Svård and Wiklund 1986; Rutowski et al. 1987). Mating history has also been found to affect fecundity and longevity in some species (Rutowski et al. 1987; Oberhauser 1988; Watanabe 1988) but not in others (Greenfield 1982; Jones et al. 1986; Svård and Wiklund 1988; Ward and Landolt 1995).

We examined the effects of male mating history and intermating interval on (1) ejaculate size, (2) copulation duration, (3) female fecundity, and (4) female longevity.

### Study species

*J. evagoras* (Donovan) is a multivoltine lycaenid butterfly that inhabits both coastal and inland regions of eastern Australia, ranging from Melbourne, Victoria, in the south, to Gladstone, Queensland, in the north (Common and Waterhouse 1981). Although widespread in distribution it tends to occur in discrete, highly localized populations, and is known to feed on at least 18 species of *Acacia* (Pierce et al. 1987). Males and females show almost no sexual dimorphism in wing color or pattern (Common and Waterhouse 1981; Pierce 1984). Ants of the genus *Iridomyrmex* not only protect *J. evagoras* larvae from predators and parasitoids, their presence also shortens larval development time (Pierce et al. 1987). In return, the larvae secrete carbohydrates and amino acids for the ants (Pierce 1983). Further details about the general biology of this species can be found in Kitching (1983), Pierce (1983, 1984, 1987), Pierce and Elgar (1985), and Pierce et al. (1987).

## Methods

*J. evagoras* butterflies were raised from eggs collected from *Acacia melanoxylon* at Mount Nebo, Queensland, Australia (152°47' E, 27°23' S). Larvae were reared until the third instar in plastic boxes on cuttings of young *A. melanoxylon* and *A. decurrens* leaves. They were then transferred to potted *A. melanoxylon* plants in a glasshouse (day temperature approximately 25°C, night temperature 21°C, 60% relative humidity, photoperiod 14:10), where they were tended by a colony of *Iridomyrmex anceps*. Once the larvae pupated, they were removed from the trees and placed in individual plastic cups. Following eclosion, butterflies were sexed, weighed, and marked with a unique number. Butterflies were then placed in cages separated by sex and fed at least once daily on a 20% honey-water solution. Male butterflies were randomly allocated into groups to be mated once, twice, three, or four times.

### Effects of mating history on ejaculate mass

Most females were mated on the day of eclosion but a few were mated 1–3 days later. Virgin females were placed in a refrigerator for 10 min to temporarily immobilize them and were then intro-

duced into a cage of at least ten males while being gently held with flat-bladed forceps around their wings. This method was necessary because female *J. evagoras* that have eclosed for more than an hour vigorously repel attempts by males to mate. Once a female was introduced in this way, males typically began to cluster around her. Once a male started to mate with the female and the female regained mobility, the pair was removed to a separate cage. As soon as copulation ended, the male was placed in a cage of other males with the same mating history. The female was killed by freezing, and dissected. The bursa copulatrix was removed and weighed to the nearest 0.01 mg on a Cahn electrobalance, then dried to constant mass and reweighed. Previous studies (Rutowski et al. 1983; Svård and Wiklund 1986), have indicated that the weight of the empty bursa is negligible, so the weights obtained (hereafter referred to as 'ejaculate') consisted of the spermatophore plus any accessory substances transferred by the male. Males were then remated 1–9 days later and the dissection procedure repeated. Seven males were remated after 1 day, 8 after 2 days, 2 after 3 days, 8 after 5 days and 2 after 9 days.

### Effect of mating history on copulation duration

Matings were conducted as described above and the length of copulation recorded to the nearest 5 min. Where possible, individual males were mated at least three times, with the time between matings varying from 1 to 5 days. Copulation duration was recorded for a total of 78 males. Of these, 44 were mated twice, 17 were mated three times, and 3 were mated four times.

### Effect of mating history on female fecundity and longevity

Matings were conducted as above except that once copulation had finished, females were placed in separate plastic oviposition cages containing feeders of honey-water solution, a sprig of *A. decurrens* foliage, and pieces of stem into which several grooves had been cut. Most female *J. evagoras* preferentially laid their eggs into these grooves. The total number of eggs the female laid and her longevity were recorded. When the females died they were dissected and any chorionated eggs remaining in their abdomens were counted.

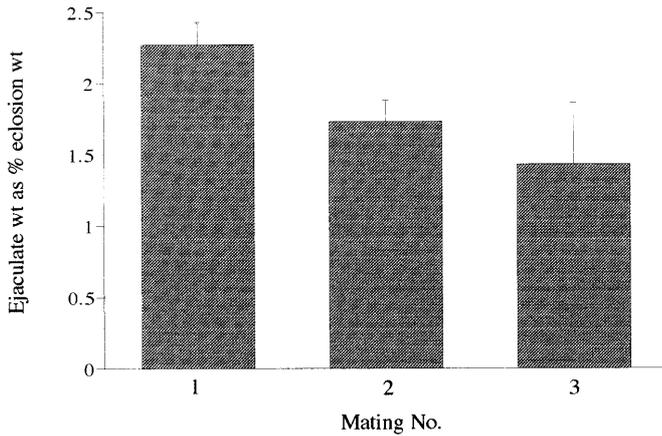
### Statistical analysis

We initially tested the effect of female and male size, female and male age, and male mating history on ejaculate size, copulation duration, female fecundity, and female longevity, using simple regressions. The significance of the analyses was tested by one-way ANOVA. Where two or more factors were both significant predictors on their own, we also tested them together in a multiple regression. Paired *t*-tests were used to test the effect of mating history (first vs second mating) on ejaculate mass. The effect of mating history on copulation duration, female fecundity, and longevity within groups of males was tested by repeated-measures ANOVA. Tukey's tests were used to compare differences in means. Data were transformed where appropriate to improve normality. All percentage data were arcsine transformed.

## Results

### Effects on ejaculate size

During the first mating, males transferred an average of 2.2% of their eclosion weight to females (range 0.9–4.0%) (Fig. 1). The average water content of the ejaculate was 43.6% ( $\pm 15\%$ ). The slope of the relationship between log male eclosion weight and log ejaculate



**Fig. 1** Ejaculate weight as a percentage of male eclosion weight at first, second, and third mating (mean±SE)

wet weight was not significantly different from 1, indicating that wet ejaculate weight increased proportionally with increasing male size (slope±95% confidence limit=0.933±0.365,  $r^2=0.451$ ,  $F_{1,31}=27.24$ ,  $P<0.0001$ ; Fig. 2a). However, the dry weight of the ejaculate was proportionally larger in smaller males (slope=0.595±0.265,  $r^2=0.341$ ,  $F_{1,37}=20.66$ ,  $P<0.0001$ ; Fig. 2a). The log dry weight of the ejaculate was positively related to male age ( $r^2=0.192$ ,  $F_{1,36}=9.81$ ,  $P=0.0034$ ; Fig. 2b). Log male weight and male age together explained 42.4% of the variation in log dry spermatophore weight ( $F_{2,34}=14.20$ ,  $P<0.0001$ ). Log wet weight of the ejaculate was not significantly related to male age. Neither wet nor dry ejaculate weight was significantly affected by female weight.

At the second mating, the wet weight of the ejaculate was, on average, 26.6% smaller than at the first mating and represented 1.7% of the males' eclosion weight (paired  $t$ -test  $t=6.558$ ,  $P<0.0001$ ,  $df=21$ ; Fig. 1). The dry weight of the ejaculate was 29.1% smaller than at the first mating ( $t=6.192$ ,  $P<0.0001$ ,  $df=27$ ; Fig. 1). The average water content of the second ejaculate was 39.7% (±17.2%). As in the first matings, the log wet weight of the ejaculate increased proportionally with log male eclosion weight but the log dry weight of the ejaculate was proportionally larger in smaller males (log wet weight: slope ±95% confidence limit=0.661±0.485,  $r^2=0.209$ ,  $F_{1,25}=7.87$ ,  $P=0.0096$ ; dry weight slope=0.275±0.313,  $r^2=0.080$ ,  $F_{1,25}=3.25$ ,  $P=0.0833$ ; Fig. 2c). Log wet weight was positively related to male age ( $r^2=0.129$ ,  $F_{1,25}=4.84$ ,  $P=0.0373$ ; Fig. 2d), and log female eclosion weight ( $r^2=0.332$ ,  $F_{1,16}=9.46$ ,  $P=0.0072$ ; Fig. 2e). Log male weight, log female weight, and male age together explained 67.3% of the variation in log wet spermatophore weight ( $F_{3,13}=12.0$ ,  $P=0.0005$ ). Log dry weight was positively related to male age ( $r^2=0.163$ ,  $F_{1,25}=6.08$ ,  $P=0.0209$ ; Fig. 2d) and time between matings ( $r^2=0.212$ ,  $F_{1,25}=7.98$ ,  $P=0.0092$ ; Fig. 2f). Log male weight, male age, and intermating interval together explained 41.8% of the variation in log dry spermatophore weight ( $F_{3,23}=7.23$ ,  $P=0.0014$ ).

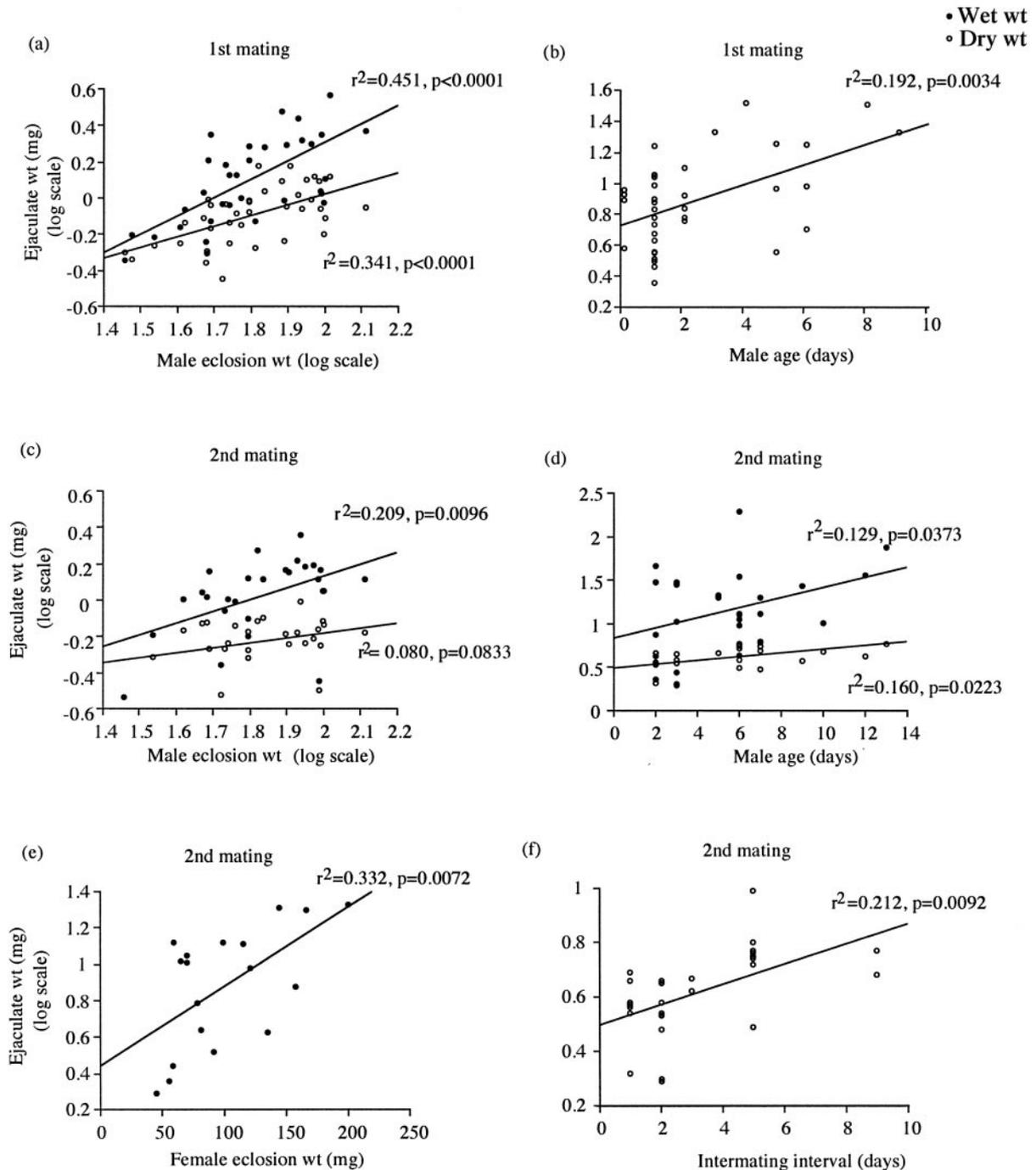
As the time between the first and second matings increased, the reduction in ejaculate weight between matings declined. Figure 3a,b shows the percentage reduction in ejaculate weight as a function of intermating interval and indicates that ejaculate weight returned to its original level 5–9 days after the first mating (wet weight:  $r^2=0.528$ ,  $F_{1,19}=23.33$ ,  $P=0.0001$ , Fig. 3a; dry weight:  $r^2=0.255$ ,  $F_{1,25}=9.92$ ,  $P=0.0042$ , Fig. 3b). To test whether large males 'recovered' at a different rate to small males, we divided the males into two size categories above and below the median wing length of 20 mm, and compared the regression slopes of percentage ejaculate weight reduction against intermating interval. There was no significant difference in slopes, indicating that male size did not affect the recovery rate of the ejaculate [wet weight: slope±95% confidence limit=−9.63±4.80 (small males,  $n=10$ ), −6.73±4.21 (large males,  $n=16$ ); dry weight: −3.289±5.13 (small males,  $n=15$ ), −5.084±3.62 (large males,  $n=22$ )]. We also tested the effect of male age on recovery rate by dividing the males into two age categories, 'young males' (aged 5 days or less at the second mating) and 'old males' (aged more than 5 days at the second mating). The slopes of the regressions of percentage decrease in ejaculate weight against intermating interval were not significantly different between the two age categories, indicating that male age did not affect recovery rate [wet weight: slope=−0.855±19.572 (young males,  $n=11$ ), −8.733±8.368 (old males,  $n=10$ ); dry weight: −14.360±11.782 (young males,  $n=12$ ), −5.135±5.532 (old males,  $n=15$ )].

We obtained ejaculate weights for three males that mated three times. These males transferred an average of 1.4% of their eclosion weight to females (Fig. 1).

#### Effects on copulation duration

The main influence on copulation duration was the number of times the male had previously mated (Fig. 4). Average copulation time increased from 2.4 h at the first mating ( $n=78$ ) to 3.1 h at the second ( $n=44$ ), 4.0 h at the third ( $n=17$ ), and 4.3 at the fourth ( $n=3$ ), (repeated-measured ANOVA for  $n=17$  three-times mated males,  $F_{2,32}=11.434$ ,  $P<0.001$ ). Within the group of males that had mated three times, copulation duration was not significantly different between once- and twice-mated males, but was significantly longer for males that had mated three times (Tukey's test,  $P<0.05$ ).

The only other factor to significantly influence copulation duration was male birth weight which was negatively associated at both the first and second, but not subsequent, matings. The amount of variation explained by this factor, however, was small (first mating:  $r^2=0.065$ ,  $F_{1,74}=6.20$ ,  $P=0.015$ ; second mating:  $r^2=0.091$ ,  $F_{1,41}=5.22$ ,  $P=0.028$ ).



**Fig. 2** Effects on ejaculate mass of male eclosion weight at first mating (log scales) (a), male age at first mating (dry weight only) (b) male eclosion weight at second mating (log scales) (c), male age at second mating (d), female eclosion weight at second mating (wet weight only) (e), and intermating interval at second mating (dry weight only) (f).  $R^2$  values are from simple regression analyses,  $P$  values are from one-way ANOVAs

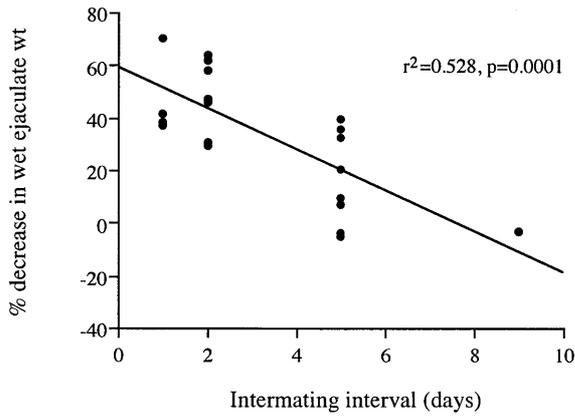
#### Effects on female fecundity

The number of eggs laid was correlated with female mass for females that mated with virgin males ( $r^2=0.11$ ,  $F_{1,26}=4.34$ ,  $P=0.0472$ , data square root transformed). Fe-

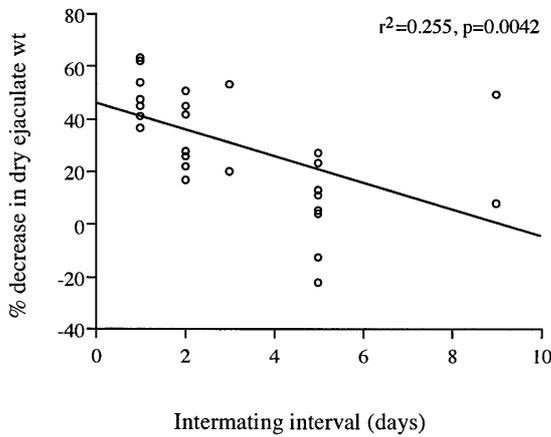
males mated with older males also produced more eggs although this was only significant at the first mating (first mating:  $r^2=0.14$ ,  $F_{1,26}=4.31$ ,  $P=0.0478$ ; second mating:  $r^2=0.14$ ,  $F_{1,15}=2.49$ ,  $P=0.1358$ ). Together, male age and female weight only accounted for 10.3% of the variation in fecundity ( $F_{2,25}=2.55$ ,  $P=0.0982$ ). No characteristics of either males or females had significant effects on fecundity for females mated to three- or four-times-mated males, probably due to the small sample sizes. There were no significant effects of copulation duration or intermating interval on fecundity.

There was a tendency for fecundity to decline with mating number although this was not statistically signifi-

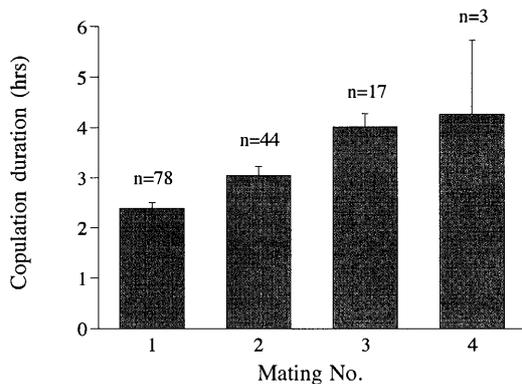
(a)



(b)

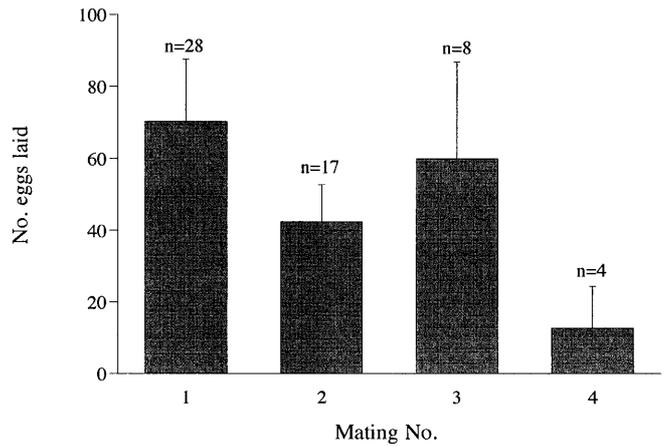


**Fig. 3** Percentage reduction in ejaculate mass as a function of intermating interval for wet weight (a) and dry weight (b)

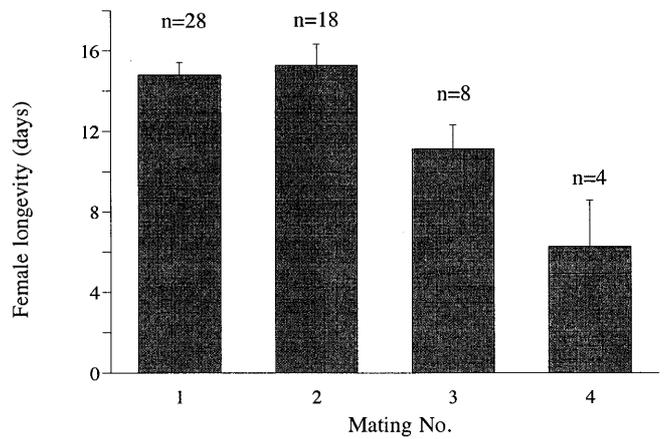


**Fig. 4** Effect of male mating number on copulation duration (mean +SE)

cant (repeated-measures ANOVA,  $F_{3,26}=2.375$ ,  $P=0.0931$ ; Fig. 5). The number of eggs laid, as a percentage of the total produced (eggs laid plus unlaid eggs dissected from the abdomen after death), was also not significantly affected by mating number ( $F_{3,26}=2.1208$ ,  $P=0.1219$ , data arcsine transformed).



**Fig. 5** Effect of male mating number on number of eggs laid (mean +SE)



**Fig. 6** Effect of male mating number on female longevity (mean +SE)

Effects on female longevity

Longevity of females generally declined as the mating number of their male mates increased (Fig. 6). There was a significant difference in longevity between females mated to once- and three-times-mated males, but not between once- and twice-mated, or twice- and three-times-mated males (repeated-measures ANOVA,  $n=8$ ,  $F_{2,14}=4.3247$ ,  $P=0.0345$ , followed by Tukey's test,  $P<0.05$ ). Longevity was not significantly affected by any other characteristics measured such as size, of either the males or females.

Discussion

The unusual mating strategy of *J. evagoras*, in which a relatively small proportion of males obtain most of the matings, imposes costs in terms of ejaculate size, copulation duration, and female longevity.

## Mass of ejaculate transferred at mating

*J. evagoras* males transferred an average of 2.2% of their eclosion weight to females during their first mating, 1.7% at the second, and 1.4% at the third. This is a relatively small amount compared to many other Lepidoptera. For example, in a comparison of ejaculate mass among ten butterfly species, Rutowski et al. (1983) found males passed an average of 6.3% of their body weight during copulation (range 4.9–8.7%). Svärd and Wiklund (1989) compared ejaculate mass for 25 species from three families and found a range from 1.4 to 15.5%. Bissoondath and Wiklund (1996b) found a range of 7.6–10.9%. Individual males have been shown to deliver up to 23% of their body weight (Forsberg and Wiklund 1989). The relatively small ejaculate of *J. evagoras*, which field observations suggest is a monandrous species and therefore not subject to sperm competition, is consistent with the hypothesis of Svärd and Wiklund (1989) and the comparative work of Karlsson (1995) that ejaculate size is positively correlated with the degree of polyandry.

The results of this study also support the related hypothesis of Rutowski et al. (1983) that the amount of material transferred during mating, relative to the size of the male, should be correlated with the degree of mate choice by both males and females in a particular species; males of species with small ejaculates should be less selective and females less aggressive in courtship and mate location than species whose males invest more. Field observations of the frenzied mating assemblage of *J. evagoras* males suggest that they do not discriminate between male and female and that females, who are mated immediately upon eclosion, do not exert any mate choice at all.

## Effects on ejaculate size

Despite the evidence that *J. evagoras* transfers a relatively small ejaculate, mating is still a significant cost, as indicated by the findings that the ejaculate decreased by nearly 30% at the second mating and that, regardless of size or age, males needed a period of 5–9 days for the ejaculate to return to approximately the same weight as at the first mating. A decline in ejaculate weight with multiple mating has been found in virtually all lepidopteran species where it has been tested (George and Howard 1968; Boggs and Gilbert 1979; Rutowski 1979, 1982, 1984b; Sims 1979; Rutowski and Gilchrist 1986; Svärd and Wiklund 1986; Rutowski et al. 1987; Oberhauser 1988; Royer and McNeil 1993; Delisle and Bouchard 1995; Bissoondath and Wiklund 1996a, 1996b; Cook and Wedell 1996; Svensson et al. 1998; Watanabe et al. 1998; Wedell and Cook 1999). Our results are also consistent with the general pattern in butterflies that males may remate before the spermatophore has 'recovered' (Sims 1979; Boggs 1981; Svärd 1985; Rutowski and Gilchrist 1986; Svärd and Wiklund 1986, 1989; Oberhauser 1988, 1992; Kaitala and Wiklund 1995; Bissoondath and Wiklund 1996a). Thornhill and Alcock (1983) have suggested that this 'leave no stone unturned'

mating strategy, in which males mate whenever they can, rather than waiting until they can transfer the maximum amount of ejaculate, should be favored when the chance of remating is low, despite depletion of accessory gland material. Our finding that *J. evagoras*, despite the relatively small male investment, nevertheless takes 5–9 days to return to the original ejaculate size, is consistent with several other studies on monandrous species (e.g., Svärd 1985; Svärd and Wiklund 1989; Bissoondath and Wiklund 1996b; Karlsson 1996).

Apart from mating history, male size was the most important influence on ejaculate size in *J. evagoras*. A similar relationship between male size and ejaculate size has been found in several other lepidopterans (Boggs 1981; Greenfield 1982; Jones et al. 1986; Rutowski and Gilchrist 1986; Svärd and Wiklund 1986, 1989; Forsberg and Wiklund 1989; Oberhauser 1989; Bissoondath and Wiklund 1995, 1996a; Hiroki and Obara 1997; Svensson et al. 1998; Watanabe et al. 1998; but see Svärd 1985; Marshall and McNeil 1989). We also found that while the wet weight of the ejaculate increased proportionally with increasing male size, the dry weight transferred was relatively larger in smaller males. Our results are therefore in partial agreement with the hypothesis of Rutowski (1984a) that all males, regardless of size, deliver a typical amount of material, with small males therefore passing a greater proportion of their body mass to achieve this quantity.

There was a trend for ejaculate mass to increase with the age of the male, although age was not as important a predictor as eclosion weight. A positive relationship between ejaculate size and age has been found in several other lepidopteran species (Sims 1979; Jones et al. 1986; Oberhauser 1988; Delisle and Bouchard 1995; Hiroki and Obara 1997; Watanabe et al. 1997; Wedell and Cook 1999; but see Svärd 1985; Royer and McNeil 1993). Increasing spermatophore size with male age may be due to the daily periodic release of sperm from the testis and its accumulation in the duplex (e.g., Giebultowicz et al. 1988). Older males have also had longer to feed and so build up resources that can be used in manufacturing accessory gland secretions.

In general, ejaculate mass in *J. evagoras* was not affected by characteristics of the female, except that, rather curiously, the wet weight of the ejaculate increased significantly with female eclosion weight at the second mating. Boggs (1981) found a positive effect of female wing length on spermatophore size in *Dryas julia* and suggested a possible reason is that larger females have a larger bursa that can receive larger spermatophores. Males of the moth *Plodia interpunctella* also produce a larger ejaculate when mating with larger females (Gage 1998). In most species, however, female characteristics have been found to have no discernible effect on the amount of ejaculate transferred (Rutowski 1984b).

## Copulation duration

Copulation duration in the Lepidoptera has been reviewed by Scott (1972), who showed that times vary

from about 10 min to several days, with one to several hours being the most common. *J. evagoras* is typical of this pattern, with the average copulation of virgin *J. evagoras* males being 2.4 h. Copulation times measured in the field for *J. evagoras* are somewhat longer (mean =  $4.32 \pm 1.8$  h,  $n=41$ ; Elgar and Pierce 1988), although these times were an average of all matings observed, at least some of which were presumably not first matings. Copulation duration increased to approximately 3 h at the second mating and to over 4 h at the third and fourth. Increasing time spent in copula with increasing mating number has been found in several other butterfly species (Rutowski and Gilchrist 1986; Svård and Wiklund 1986; Rutowski et al. 1987; Bissoondath and Wiklund 1996a; Watanabe et al. 1998; Wiklund et al. 1998).

Lengthy copulation times have generally been interpreted as a mechanism to increase male fitness by reducing the probability of further mating by the female (Parker 1970). However, in a species such as *J. evagoras* where the female mates only once, and where sperm displacement is presumably not an issue, the adaptive nature of prolonged copulation is not clear, especially as it imposes costs on both sexes by exposing the copulating pair to increased risk of predation, and by taking time that could otherwise be spent mating again (for the male), or for feeding and ovipositing (for the female). This may be an especially serious cost to females, which live only about 3 days in the field (Elgar and Pierce 1988). It therefore seems likely that the increasing copulation times in *J. evagoras* are simply due to the physical constraints of passing ejaculate (Rutowski and Gilchrist 1986).

#### Effects on fecundity

Male age at first mating was positively associated with fecundity but accounted for only about 14% of variation. We did not find a significant effect of mating history on realized fecundity in *J. evagoras*. The effect on female fecundity of the depletion in ejaculate with successive matings is controversial. Radiotracer studies in several lepidopterans have shown that accessory gland secretions passed into the female's reproductive tract during mating are absorbed by the female and used in egg production (Boggs and Gilbert 1979; Boggs 1981; Boggs and Watt 1981; Greenfield 1982; Wiklund et al. 1993; Wedell 1996). Some studies have demonstrated increases in fecundity with increasing ejaculate size (Rutowski et al. 1987; Royer and McNeil 1993; Foster and Ayres 1996; Karlsson 1998; Wiklund et al. 1998), while others have not (Greenfield 1982; Jones et al. 1986; Svård and Wiklund 1988, 1991; Fitzpatrick and McNeil 1989; Lederhouse et al. 1989; Delisle and Bouchard 1995; Ward and Landolt 1995; Hiroki and Obara 1997). Oberhauser (1997) noted that most species in which spermatophore depletion has been shown to affect fecundity are polyandrous, whereas the effect has rarely been found for monandrous species. Our results for *J. evagoras*, a monandrous species, are consistent both with this observation

and with the model proposed by Boggs (1990), predicting that for species in which most eggs are already yolked when females eclose, and when the male spermatophore is relatively small, and adults are fed on dilute honey-water containing diverse nutrients, there will be a lack of detectable effect on female fecundity of variation in male-donated nutrients. One explanation is that despite differences in absolute size of the ejaculate at different matings, the nutrient content remains approximately constant in terms of hydrocarbons (Marshall and McNeil 1989), nitrogen (Oberhauser 1992), and protein (Bissoondath and Wiklund 1996b). Furthermore, sperm density has actually been found to increase with successive matings in some species, despite declines in ejaculate mass (Cook and Wedell 1996; Watanabe et al. 1998).

#### Effects on longevity

We found that the lifespan of female *J. evagoras* was significantly reduced as the number of previous matings of her male partner increased. This reduction in longevity was not correlated with any other factors such as copulation duration. A similar relationship between female longevity and male mating history has been found by Rutowski et al. (1987), but not in several other studies where it has been tested (Svård and Wiklund 1991; Royer and McNeil 1993; Ward and Landolt 1995; Svensson et al. 1998). The mechanism by which female lifespan is affected by male mating history is unclear but could be related to the receipt of male-derived nutrients. Increased longevity of females in male-donating species has been documented in several butterflies (e.g., Rutowski and Gilchrist 1986; Wiklund et al. 1993, 1998; Tamhankar 1995; Wedell 1996; Rogers and Marti 1997; Karlsson 1998). If such nutrients are advantageous to females, then their possible depletion in the ejaculate of multiply mated males may have a negative effect.

The correlation between female longevity and mating history in the laboratory may not, however, be translated to non-laboratory populations, as field observations indicate that females rarely live more than 3 days.

#### Conclusions

The close association of *J. evagoras* with ants in the larval and pupal stage, leading to highly localized aggregations of fertilizable females, may have been a selective influence favoring an explosive mating system, characterized by a lack of active mate choice, lack of discrimination among mates, female monogamy, and multiple mating by males. Compared to other butterfly species, male *J. evagoras* transfer a relatively small amount of ejaculate as a proportion of their body mass, supporting the hypothesis that small ejaculates are favored in species where mate choice is lacking and female monogamy likely. However, despite the small male investment in ejaculate, the costs of multiple mating in this species

may nonetheless be significant, as indicated by the reduction in ejaculate mass, an increase in copulation duration, and reduction in female lifespan with increasing mating number. Mating history did not, however, have a significant effect on female fecundity, consistent with findings for other monandrous species.

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## References

- Bissoondath CJ, Wiklund C (1995) Protein content of spermatophores in relation to monandry/polyandry in butterflies. *Behav Ecol Sociobiol* 37:365–371
- Bissoondath CJ, Wiklund C (1996a) Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). *Funct Ecol* 10:457–464
- Bissoondath CJ, Wiklund C (1996b) Male butterfly investment in successive ejaculates in relation to mating system. *Behav Ecol Sociobiol* 39:285–292
- Boggs CL (1981) Selection pressures affecting male nutrient investment at mating in heliiconine butterflies. *Evolution* 35:931–940
- Boggs CL (1990) A general model of male-donated nutrients in female insects' reproduction. *Am Nat* 136:598–617
- Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* 206:83–84
- Boggs CL, Watt WB (1981) Population structure of pierid butterflies. IV. Genetic and physiological investment of offspring by male *Colias*. *Oecologia* 50:320–324
- Common IFB, Waterhouse DF (1981) *Butterflies of Australia*. Angus and Robertson, Sydney
- Cook PA, Wedell N (1996) Ejaculate dynamics in butterflies: a strategy for maximizing fertilization success. *Proc R Soc Lond B* 263:1047–1051
- Deinert EI, Longino JT, Gilbert LE (1994) Male competition in butterflies. *Nature* 370:23–24
- Delisle J, Bouchard A (1995) Male larval nutrition in *Choristoneura rosaceana* (Lepidoptera: Tortricidae): an important factor in reproductive success. *Oecologia* 104:508–517
- Elgar MA, Pierce NE (1988) Mating success and fecundity in an ant-tended butterfly. In: Clutton-Brock TH (eds) *Reproductive success: studies of selection and adaptation in contrasting breeding systems*. Chicago University Press, Chicago, pp. 59–75
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215–223
- Fitzpatrick SM, McNeil JN (1989) Lifetime mating potential and reproductive success in males of the true armyworm, *Pseudaletia unipuncta* (Haw.) (Lepidoptera: Noctuidae). *Funct Ecol* 3:37–44
- Forsberg J, Wiklund C (1989) Mating in the afternoon: time-saving in courtship and remating by females of a polyandrous butterfly, *Pieris napi* L. *Behav Ecol Sociobiol* 25:349–356
- Foster SP, Ayres RH (1996) Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). *J Insect Physiol* 42:657–667
- Gage MJG (1998) Influences of sex, size, and symmetry on ejaculate expenditure in a moth. *Behav Ecol* 9:592–597
- George JA, Howard MG (1968) Insemination without spermatophores in the oriental fruit moth, *Grapholitha molesta* (Lepidoptera: Tortricidae). *Can Entomol* 100:190–192
- Giebułtowicz JM, Bell RA, Imberski RB (1988) Circadian rhythm of sperm movement in the male reproductive tract of the gyp moth, *Lymantria dispar*. *J Insect Physiol* 34:527–532
- Greenfield MD (1982) The question of paternal investment in Lepidoptera: male-contributed proteins in *Plodia interpunctella*. *Int J Invert Reprod* 5:323–330
- Hiroki M, Obara Y (1997) Delayed mating and its cost to female reproduction in the butterfly, *Eurema hecabe*. *J Ethol* 15:79–85
- Jones KN, Odendaal FJ, Ehrlich PR (1986) Evidence against the spermatophore as paternal investment in checkerspot butterflies (*Euphydryas*: Nymphalidae). *Am Midl Nat* 116:1–6
- Kaitala A, Wiklund C (1995) Female mate choice and mating costs in the polyandrous butterfly *Pieris napi* (Lepidoptera: Pieridae). *J Insect Behav* 8:355–363
- Karlsson B (1995) Resource allocation and mating systems in butterflies. *Evolution* 49:955–961
- Karlsson B (1996) Male reproductive reserves in relation to mating system in butterflies: a comparative study. *Proc R Soc Lond B* 263:187–192
- Karlsson B (1998) Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. *Ecology* 79: 2931–2940
- Kitching RL (1983) Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly *Jalmenus evagoras* (Donovan). *J Nat Hist* 17:471–481
- Lederhouse RC, Ayres MP, Scriber JM (1989) Evaluation of spermatophore counts in studying mating systems in the Lepidoptera. *J Lepid Soc* 43:93–101
- Marshall LD, McNeil JN (1989) Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). *Funct Ecol* 3:605–612
- Oberhauser KS (1988) Male monarch butterfly spermatophore mass and mating strategies. *Anim Behav* 36:1384–1388
- Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. *Behav Ecol Sociobiol* 25:237–246
- Oberhauser KS (1992) Rate of ejaculate breakdown and intermating intervals in monarch butterflies. *Behav Ecol Sociobiol* 31:367–373
- Oberhauser KS (1997) Fecundity, lifespan and egg mass in butterflies: effects of male-derived nutrients and female size. *Funct Ecol* 11:166–175
- Odendaal FJ, Iwasa Y, Ehrlich PR (1985) Duration of female availability and its effect on butterfly mating systems. *Am Nat* 125:673–678
- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567
- Pierce NE (1983) The ecology and evolution of symbioses between lycaenid butterflies and ants. PhD thesis. Harvard University, Cambridge, Mass
- Pierce NE (1984) Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. *Symp R Entomol Soc Lond* 11:197–200
- Pierce NE (1987) The evolution and biogeography of associations between lycaenid butterflies and ants. In: Harvey PH, Partridge L (eds) *Oxford surveys in evolutionary biology*. Oxford University Press, Oxford, pp 89–116
- Pierce NE, Elgar MA (1985) The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behav Ecol Sociobiol* 16:209–222
- Pierce NE, Kitching RL, Buckley RC, Taylor MFJ, Benbow KF (1987) The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behav Ecol Sociobiol* 21:237–248
- Rogers CE, Marti OG Jr (1997) Once-mated beet armyworm (Lepidoptera: Noctuidae): effects of age at mating on fecundity, fertility and longevity. *Environ Entomol* 26:585–590
- Royer L, McNeil JN (1993) Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. *Funct Ecol* 7:209–215
- Rutowski RL (1979) The butterfly as an honest salesman. *Anim Behav* 27:1269–1270
- Rutowski RL (1982) Mate choice and Lepidopteran mating behavior. *Fla Entomol* 65:72–82

- Rutowski RL (1984a) Sexual selection and the evolution of butterfly mating behavior. *J Res Lepid* 23:125–142
- Rutowski RL (1984b) Production and use of secretions passed at copulation by males in *Pieris protodice* (Lepidoptera: Pieridae). *Psyche* 91:141–152
- Rutowski RL (1991) The evolution of male mate-locating behavior in butterflies. *Am Nat* 138:1121–1139
- Rutowski RL, Gilchrist GW (1986) Copulation in *Colias eurytheme* (Lepidoptera: Pieridae): patterns and frequency. *J Zool Lond A* 209:115–124
- Rutowski RL, Newton M, Schaefer J (1983) Interspecific variation in the size of the nutrient investment made by male butterflies during copulation. *Evolution* 37:708–713
- Rutowski RL, Gilchrist GW, Terkanian B (1987) Female butterflies mated with recently mated males show reduced reproductive output. *Behav Ecol Sociobiol* 20:319–322
- Rutowski RL, Gilchrist GW, Terkanian B (1988) Male mate-locating behavior in *Euphydryas chalcedona* (Lepidoptera: Nymphalidae) related to pupation site preferences. *J Insect Behav* 1:277–289
- Scott JA (1972) Mating of butterflies. *J Res Lepid* 11:99–127
- Scott JA (1974) Mate-locating behavior of butterflies. *Am Midl Nat* 91:103–117
- Silberglied RE (1977) Communication in the Lepidoptera. In: Seebeek TA (ed) *How animals communicate*. Indiana University Press, Bloomington, pp. 362–402
- Sims SR (1979) Aspects of mating frequency and reproductive maturity in *Papilio zelicaon*. *Am Midl Nat* 102:36–50
- Smiley JT, Atsatt PR, Pierce NE (1988) Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. *Oecologia* 76:416–422
- Svärd L (1985) Paternal investment in a monandrous butterfly, *Parage aegeria*. *Oikos* 45:66–70
- Svärd L, Wiklund C (1986) Different ejaculate delivery strategies in first versus subsequent matings in the swallowtail butterfly *Papilio machaon* L. *Behav Ecol Sociobiol* 18:325–330
- Svärd L, Wiklund C (1988) Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly. *Behav Ecol Sociobiol* 23:39–43
- Svärd L, Wiklund C (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behav Ecol Sociobiol* 24:395–402
- Svärd L, Wiklund C (1991) The effect of ejaculate mass on female reproductive output in the European swallowtail butterfly, *Papilio machaon* (L.) (Lepidoptera: Papilionidae). *J Insect Behav* 4:33–42
- Svensson MGE, Marling E, Lofqvist J (1998) Mating behavior and reproductive potential in the turnip moth *Agrotis segetum* (Lepidoptera: Noctuidae). *J Insect Behav* 11:343–359
- Tamhankar AJ (1995) Host influence on mating behavior and spermatophore reception correlated with reproductive output and longevity of female *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae). *J Insect Behav* 8: 499–511
- Thornhill R, Alcock J (1983) *The evolution of insect mating systems*. Harvard University Press, Cambridge, Mass
- Ward KE, Landolt PJ (1995) Influence of multiple matings on fecundity and longevity of female cabbage looper moths (Lepidoptera: Noctuidae). *Ann Entomol Soc Am* 88:768–772
- Watanabe M (1988) Multiple matings increase the fecundity of the yellow swallowtail butterfly, *Papilio xuthus* L., in summer generations. *J Insect Behav* 1:17–29
- Watanabe M, Nakanishi Y, Bon'no M (1997) Prolonged copulation and spermatophore size ejaculated in the sulphur butterfly, *Colias erate* (Lepidoptera: Pieridae) under selective harassments of mated pairs by conspecific lone males. *J Ethol* 15:45–54
- Watanabe M, Wiklund C, Bon'no M (1998) The effect of repeated matings on sperm numbers in successive ejaculates of the cabbage white *Pieris rapae* (Lepidoptera: Pieridae). *J Insect Behav* 11:559–570
- Wedell N (1996) Mate quality affects reproductive effort in a paternally investing species. *Am Nat* 148:1075–1088
- Wedell N, Cook PA (1999) Strategic sperm allocation in the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae). *Funct Ecol* 13:85–93
- Wiklund C, Kaitala A, Lindfors V, Abenius J (1993) Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). *Behav Ecol Sociobiol* 33:25–33
- Wiklund C, Kaitala A, Wedell N (1998) Decoupling of reproductive rates and parental expenditure in a polyandrous butterfly. *Behav Ecol* 9:20–25