Mating system and the evolution of sex-specific mortality rates in two nymphalid butterflies

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Life-history theory predicts that organisms should invest resources into intrinsic components of lifespan only to the degree that it pays off in terms of reproductive success. The benefit of a long life may differ between the sexes and different mating systems may therefore select for different sex-specific mortality rates. In insects with polyandrous mating systems, females mate throughout their lives and male reproductive success is likely to increase monotonously with lifespan. In monandrous systems, where the mating season is less protracted because receptive females are available only at the beginning of the flight season, male mating success should be less dependent on a long lifespan. Here, we show, in a laboratory experiment without predation, that the duration of the mating season is longer in the polyandrous comma butterfly, *Polygonia c-album*, than in the monandrous peacock butterfly, *Inachis io*, and that, in line with predictions, male lifespan is shorter than female lifespan in *I. io*, whereas male and female lifespans are similar in *P. c-album*.

Keywords: life history; ageing; polygamy; monandry; polyandry; senescence

1. INTRODUCTION

A major challenge in life-history theory is to explain how and why fundamental traits that are closely related to fitness vary between organisms. Explanations are based on the notion that natural selection will maximize individual fitness within the constraints set up by the trade-offs and genetic variation present in each specific case (Stearns 1992; Roff 2002). Therefore the exact combination of life-history traits that satisfy this criterion varies between organisms and across mating systems. Realized adult longevity is a crucial life-history trait as it will influence how many times an individual can engage in reproductive activities. As a consequence, evolutionary theories of ageing are largely integrated with the general theories of life-history evolution. They suggest that ageing has evolved because external insults to survival and fecundity make the force of natural selection progressively weaker throughout the adult period (Medawar 1952; Williams 1957; Stearns 1992; Charlesworth 1994; McNamara & Houston 1996; Partridge & Barton 1996). Given that ageing occurs, its specific rate can be viewed from the perspective of general life-history optimization. Because organisms in the wild are generally killed by external factors such as predation or starvation before they grow old, they should invest resources into the intrinsic components of lifespan only to the degree that it pays off in terms of reproductive success (Kirkwood 1987; Kirkwood & Rose 1991). From this perspective it seems reasonable to assume that variation between organisms in the intrinsic capacity for longevity may reflect differences in the relative costs or benefits of a long adult life. In this study, we have investigated how variation in mating system may influence the relative benefits of a long adult lifespan. To do this we have compared sex-specific mortality patterns in two closely related butterfly species whose basic ecology and phenology are

similar but which differ in mating system, namely the monandrous peacock butterfly, *Inachis io*, and the polyandrous comma butterfly, *Polygonia c-album*.

The basic rationale for our study is that the time distribution of male mating opportunity should have a strong impact on male phenology and longevity. Essentially, males can have their germ line perpetuated over time only through reproduction, for which mating is necessary. All other things being equal, this means that selection for extended male longevity should be stronger in polyandrous mating systems in which males are allowed to mate with females throughout the female flight period than in monandrous mating systems in which male mating opportunity is considerably more restricted in time. Hence, under polyandry, male mating success, which is strongly associated with male reproductive success, is likely to increase monotonously with lifespan, because, in all polyandrous butterflies hitherto studied, females become unreceptive for a number of days after mating, and so polyandrous females distribute their matings substantially over time (Wiklund *et al.* 1993). Conversely, under monandry, male mating success should not be expected to be strongly associated with lifespan, because receptive females are available for a rather short time period, the exact timing of which depends on the life cycle. In species that overwinter as eggs, larvae or pupae, the adult butterflies typically have rather short lifespans, the females become receptive very soon after eclosion (indeed, it is hard to find unmated females in many species; Wiklund & Fagerström 1977) and the time during which receptive females are available coincides largely with the female eclosion period. In butterflies that overwinter as adults, lifespan is typically rather long (because the winter months that are spent inactive are included in the lifespan), and females become receptive after hibernation. But even so, receptive females are available for a rather short time period because these females mate very soon after becoming active in the spring (indeed, it is just as hard to find unmated post-diapause females of the adult

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overwintering *Gonepteryx rhamni* as it is to find virgin female butterflies of other species as mentioned above; Wiklund *et al.* 1996). Hence, the expected association between mating system and sex-specific mortality rate holds irrespective of butterfly life cycle and phenology, and in this paper we test the prediction that male mortality rate should be faster than female mortality rate in the monandrous *I. io*, whereas male mortality rate should not differ substantially from female mortality rate in the polyandrous *P. c-album*. Because mortality rates may differ between species for a number of reasons, our test will be to see whether there is an interaction between species and sex-specific mortality rates in these two species.

2. MATERIAL AND METHODS

(a) *Life cycles, ecology and mating systems of study species*

In Sweden *I. io* and *P. c-album* are both univoltine and overwinter in the adult stage. Although the adult butterflies eclose in the middle of summer, during July, all adults are in reproductive diapause and spend the time up to hibernation foraging and resting. Both species emerge from hibernation during April or May, and males of both species set up and defend territories along forest edges, and in both species males are observed several days before females (cf. Baker 1972; C. Wiklund, personal observation). Mating is notoriously difficult to observe in the field in these species owing to the utterly coy nature of the females; in the peacock butterfly, *I. io*, as in the small tortoiseshell, *Aglais urticae*, it is not unusual to observe courting males following females in the late afternoons in April and May, but the actual copulation appears to be preceded by an excessively extended male pursuit and seems to be initiated late in the evening (cf. Baker 1984). Hence, it is hard to assess the actual durations of the mating periods of *I. io* and *P. c-album*, but egg-laying females can be observed from late April to early June, suggesting that the duration of the post-diapause female flight period extends over some two months. Both *I. io* and *P. c-album* use the common nettle, *Urtica dioica*, as a host plant; however, whereas *I. io* is monophagous on this host plant, *P. c-album* is polyphagous and uses a variety of plants as hosts (cf. Nylin 1988).

The frequency of female mating was assessed by spermatophore counts in the bursa copulatrix of wild-caught females: at mating males transfer a single spermatophore, and so the lifetime number of matings can be determined simply by counting the number of spermatophores. The average number of female matings in *P. c-album* has previously been shown to be 2.5 (Svärd & Wiklund 1989), whereas that of *I. io* was unknown. Hence, we collected wild females of *I. io* to establish the female mating frequency for this species.

(b) *Experimental conditions*

Previous experimental work has shown that very long day lengths, exceeding 18 h, can make adult nymphalid butterflies, such as *P. c-album* and *I. io*, forego hibernation and start mating within 3 days of adult eclosion (C. Wiklund, unpublished data). Therefore we performed two sets of experiments, one experiment in which adult *P. c-album* and *I. io* butterflies were allowed to reproduce without adult diapause, and a second experiment in which they had spent a minimum of six months in hibernation at 4 °C before they were allowed to reproduce. The first experiment was carried out in the autumn of 2000, and the second

was carried out during the autumn of 2001 and the spring of 2002. The adults used in the experiments were the offspring of females that were wild-caught in the vicinity of Stockholm, Sweden, and all larvae of both species were reared on *U. dioica*.

(i) *Reproduction with no diapause*

All individuals were sexed as pupae, and individually marked with a gold marker pen at eclosion. On the day after eclosion all adults were released into one of two flight cages measuring 0.8 m × 0.8 m × 0.5 m with transparent plastic tops, in which 25% sucrose solution was provided in two plastic cups, and in which was placed a potted host plant (*U. dioica*) to allow egg-laying by females. A new potted *U. dioica* was placed in the cage every fifth day to prevent larvae that had hatched from devouring the host plant; hence all females had access to a host plant suitable for egg-laying throughout their lives. The *P. c-album* cage housed a total of 13 males and 17 females, whereas the *I. io* cage housed a total of 24 males and 22 females. The cages were lit and heated by 400 W mercury vapour lamps placed 2 m above the tops of the cages. The day length was 19 h with the lights turned on at 2230 (and turned off at 1730); this day length is approximately equal to that at the summer solstice in Sweden at the latitude of Stockholm. The reason the lights were turned on at 2230 was that previous preliminary experiments (C. Wiklund, unpublished data) had shown that matings in both species were initiated some 11–17 h after the onset of the photophase; hence, the majority of matings occurred during normal 'working hours' making it possible for us to record matings by direct observation. The cages were observed for mating pairs every 30 min between 0830 and 1730. The number of matings was also assessed by dissection of females after their natural deaths in order to count the number of spermatophores in the bursa copulatrix. All individuals were allowed to live freely in the cage, and the day of death was noted so as to assess individual lifespan.

(ii) *Reproduction after hibernation*

The *P. c-album* individuals eclosed between 25 August and 5 September 2001, whereas the *I. io* individuals eclosed between 10 and 20 October 2001. On the day of eclosion adult *P. c-album* and *I. io* were taken to one of two cages, which were identical to the ones in which the butterflies that had reproduced without diapause had been housed, under identical conditions, except that they were maintained on an 8 h day length (lights were turned on at 0900 and turned off at 1700). The butterflies were kept under these conditions for two weeks, because a couple of weeks of feeding greatly increases adult survival during hibernation (Pullin 1987). The cages were checked for eventual matings every hour, but no mating pairs were observed under these pre-diapause conditions. After a minimum of two weeks of feeding, the butterflies were individually transferred to 150 ml plastic cups inside which they were allowed to settle on a gauze mesh that was held on the top of the cup with a rubber band. The cups were placed in a plastic tray on the bottom of which a wetted newspaper was kept as a wet mat. Each plastic tray housed 42 butterflies and the butterflies were kept in the dark by means of a newspaper that covered the top of the tray. Every month about a litre of water was poured onto the bottom of the tray to maintain humidity for the hibernating butterflies. The trays were kept in a cold room at 4 °C from September until the beginning of December, when they were transferred outdoors to a shed on the roof of the Zoology Department building. The trays with the overwintering butterflies were brought back into

Table 1. Number of spermatophores in females of *Polygonia c-album* and *Inachis io*.
(Values are given both for wild-caught females and for experimental females that spent their entire lives in flight cages in the laboratory containing approximately equal numbers of males and females.)

number of spermatophores		0	1	2	3	4	5
wild-caught	*P. c-album*	0	2	6	4	0	1
	I. io	1	25	2	0	0	0
non-diapause experiment	*P. c-album*	1	4	6	4	2	0
	I. io	5	11	3	0	0	0
hibernation experiment	*P. c-album*	2	6	15	8	2	0
	I. io	16	11	1	0	0	0

the 4 °C cold room early in March 2001, and were kept there until the middle of April when the experiments were started. Winter mortality was 5–10% for both species. Surviving butterflies were sexed and marked individually as in experiment one, and to avoid overcrowding we used two *P. c-album* cages, one holding 16 males and 17 females, the other holding 15 males and 16 females, and two *I. io* cages, one holding 12 males and 14 females, the other holding 11 males and 14 females. The conditions for these post-diapause butterflies were identical to those for the butterflies in the experiments in which they reproduced without diapause, and most importantly they were maintained under a 19 h day length. However, our observations of matings were sparser and so we relied on spermatophore counts to assess female mating frequency. Hence, mating frequencies of individual males could not be assessed. The cages were checked daily for dead butterflies so as to assess individual lifespan.

(c) *Statistical procedure*

To analyse mortality-rate patterns we used Cox proportional-hazards regression, which is one of the standard methods for analysing time-to-event data such as survival (Fox 2001). In particular, we wanted to investigate potential differences in sex-specific mortality rates between species. The presence of such a difference is indicated by a significant effect of the interaction between sex and species on mortality rate. Because we followed all individuals until death we had no censored observations in the analysis, which was performed with STAT v. 7.0 for Macintosh.

In all cases where we calculated the degree of protandry from spermatophore counts we excluded females that were unmated.

3. RESULTS

In total we measured adult lifespan and mating frequency in 30 individuals of *P. c-album* (17 females and 13 males) and 46 individuals of *I. io* (22 females and 24 males) among the non-diapausing butterflies. Four of the *I. io* adults (three females and one male) were in reproductive winter diapause and were therefore excluded from the analysis. Among the hibernating groups of butterflies we measured lifespan and mating frequency in 64 individuals of *P. c-album* (31 males and 33 females) and 51 individuals of *I. io* (23 males and 28 females).

(a) *Mating frequency*

Spermatophore counts in wild-caught females showed that the lifetime number of matings was higher in *P. c-album* than in *I. io* (table 1; mean number of matings ± 1 s.e. for females: *P. c-album* 2.38 ± 0.31, $n = 13$; *I. io*

1.07 ± 0.05, $n = 27$; Mann–Whitney test for difference between species: $p < 0.001$). Hence, *P. c-album* can be considered polyandrous in the wild, whereas *I. io* can be categorized as essentially monandrous. This life-history difference between the two species was upheld in the laboratory experiments. In the non-diapause experiment the mating frequencies of mated females as assessed by spermatophore counts showed that the lifetime number of matings performed by female *P. c-album* was 2.25 ± 0.25, whereas that of *I. io* was 1.21 ± 0.11 (table 1; Mann–Whitney test for difference between species: $p < 0.001$); the corresponding values in the hibernation experiment were 2.19 ± 0.15 for *P. c-album*, and 1.08 ± 0.08 for *I. io* (table 1; Mann–Whitney test for difference between species: $p < 0.001$).

In the non-diapause experiment the mating frequencies of males were estimated by counting the observed matings between 0830 and 1730. In total we observed 29 matings for *P. c-album*, all starting between 0915 and 1530 (21 of which terminated after the lights had been turned off at 1730), whereas we observed 15 matings for *I. io*, all starting between 1055 and 1430 (five of which terminated after the onset of the scotophase). Comparing these figures with the spermatophore counts shows that we missed seven matings for *P. c-album* and two for *I. io*. Hence, the data for males are somewhat imperfect, but, taking into account only observed matings, male *P. c-album* mated more often than male *I. io* (*P. c-album* 2.3 ± 0.31, $n = 13$; *I. io* 0.65 ± 0.19, $n = 23$; Mann–Whitney test for difference between species: $p < 0.001$).

Based on our observations in the non-diapause experiment, matings occurred between days 3 and 11 in *I. io*, and between days 1 and 21 in *P. c-album*; hence the duration of the mating period was approximately twice as long in the polyandrous *P. c-album* as in the monandrous *I. io* (figure 1; Mann–Whitney test for difference between species: $p < 0.05$).

(b) *Species differences in sex-specific mortality*

Individual adult lifespan in the non-diapause experiment ranged from 21 to 45 days in *P. c-album* and between 9 and 29 days in *I. io*; the corresponding values for post-diapause lifespan were 22–45 days in *P. c-album* and 8–32 days in *I. io* (cf. means ± 1 s.e. in tables 2 and 3). The analysis indicated that both species and the interaction between species and sex significantly affected mortality rate (Cox regression for species in the non-diapause experiment: Wald = 44.8, $p < 0.0001$; sex: Wald = 0.051, $p = 0.82$; interaction: Wald = 7.4, $p = 0.007$, d.f. = 1 in all cases; the corresponding Cox regression for species in the
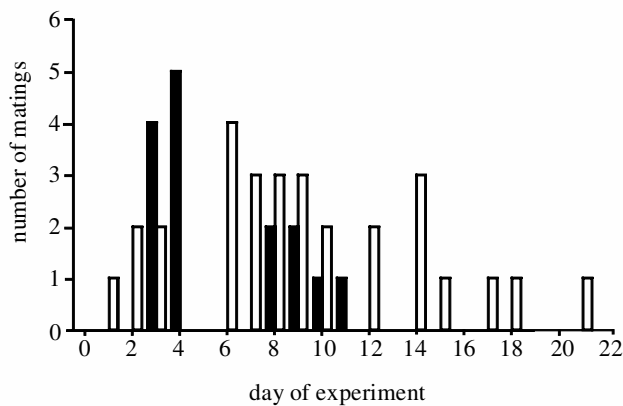
Figure 1. The time distribution of observed matings by *Polygonia c-album* (open bars) and *Inachis io* (filled bars) in the non-diapause experiment, in which the butterflies were maintained at a 19 h day length and started mating and reproduction without prior diapause.

Table 2. Sex-specific lifespans of the two species (mean ± 1 s.e. days) for individuals reproducing with no diapause (without hibernation).

Polygonia c-album		Inachis io	
females	males	females	males
30.2 ± 1.7	31.0 ± 1.2	19.6 ± 1.4	13.3 ± 0.6
$n = 17$	$n = 13$	$n = 19$	$n = 23$

Table 3. Sex-specific post-diapause lifespans of the two species (mean ± 1 s.e. days) for individuals that have overwintered before reproducing.
(On average *Polygonia c-album* individuals had had a pre-reproductive lifespan of 230 days (hence had emerged some seven months prior to the experiments); the corresponding pre-diapause lifespan of *Inachis io* individuals was 185 days.)

Polygonia c-album		Inachis io	
females	males	females	males
31.5 ± 1.0	32.2 ± 1.0	17.9 ± 1.4	12.1 ± 0.6
$n = 33$	$n = 31$	$n = 28$	$n = 23$

hibernation experiment: Wald = 37.7, $p < 0.0001$; sex: Wald = 0.35, $p = 0.55$; interaction: Wald = 14.9, $p < 0.0001$, d.f. = 1 in all cases). In line with predictions, the significant interaction was caused by the females of *I. io* having a lower mortality rate than the males, whereas no such sex difference was present in *P. c-album* (figure 2).

4. DISCUSSION

The results show that the time distribution of matings is less protracted in the monandrous *I. io* than in the polyandrous *P. c-album*, and that the mortality rate is sex-specific in *I. io*, with males having a much shorter lifespan than females, whereas there seems to be no such difference in mortality rate between the sexes in *P. c-album*. This result is in accordance with our predictions based on the rationale that selection for a long adult life should be stronger among males in polyandrous species where

females mate repeatedly throughout their lives and in which the average male lifetime number of matings increases monotonously with lifespan.

The underlying rationale behind our prediction that a mating system can exert a selective pressure on sex-specific mortality rate, which translates to longevity, is that male reproductive success is strongly coupled with mating opportunity, which can be limited in time in species where receptive females are available only for a short period, whereas female reproductive success is linked with fecundity, which is expected to increase monotonously with longevity. Based upon the same underlying rationale we have shown earlier that sex-specific mortality rates in the monandrous *P. aegeria* vary between populations. In this species males have a higher mortality rate, and hence a much shorter lifespan, than females in temperate seasonal populations, where the temporal availability of receptive females coincides with the relatively short eclosion period, while there is no difference in mortality rates in more tropical non-seasonal populations, where receptive females are available throughout the year with no obvious temporal pattern (Gotthard *et al.* 2000).

In essence, our present understanding of the earlier emergence of males compared with females, known as protandry, is based on similar reasoning—that the temporal occurrence of males should be an adaptive response to that of female availability. Mathematical models show that male mating opportunity is in general maximized by emerging before females, with late-emerging males being more heavily penalized in the evolutionary game (because they are destined to encounter only a small number of receptive females) than early-emerging males (which risk premature death before any females have emerged, but stand the chance of encountering a large number of receptive females once they start to eclose (Wiklund & Fagerström 1977; Fagerström & Wiklund 1982; Iwasa *et al.* 1983)).

The experiments reported here were done under relatively benign laboratory conditions with non-limited availability of food and in the absence of predation, and so the mortality rate observed is in all likelihood lower than that experienced by the butterflies under natural field conditions. However, our objective here was not to assess the 'natural lifespan' of male and female *P. c-album* and *I. io*, but to assess the physiological mortality rate, which should coincide with intrinsic factors that influence the actual mortality rate experienced by the insects in the wild. It may be relevant to point out that, although both of these butterfly species overwinter before reproduction in the wild, and hence as adult individuals live for about 300 days (assuming that they eclose in mid-July and die towards the end of May), they lived for a considerably shorter time in the non-diapause experiment, most certainly as a result of our experimental manipulation, which made the butterflies start to reproduce without prior diapause. This is in agreement with a tenet of general life-history theory: that selection for extended lifespan beyond the period of reproduction should be weak.

Comparison of male and female lifespans between the two experiments shows not only that male lifespan is shorter than female lifespan in *I. io* under both conditions, and that there is no such sex-specific difference in *P. c-album*, but also that the post-diapause lifespans of the
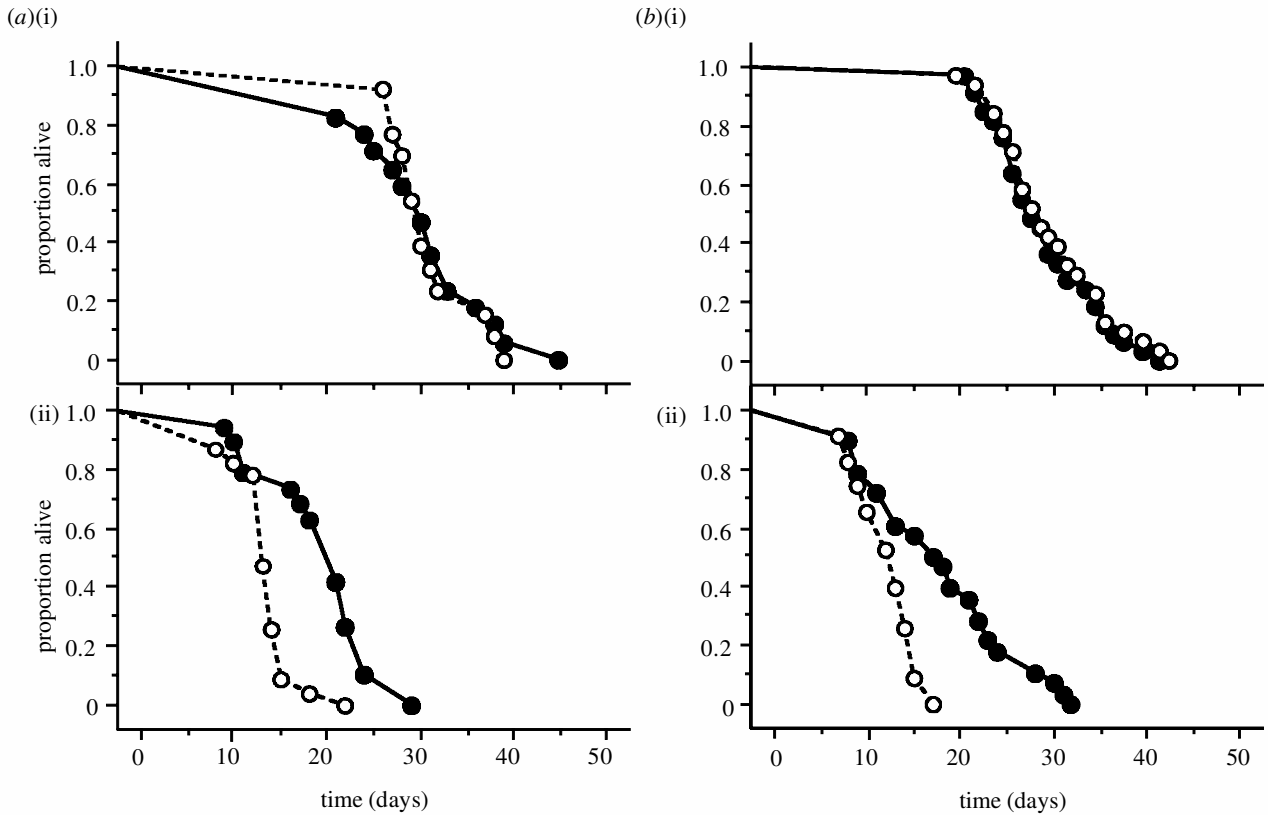
Figure 2. (*a*) Adult survival of (i) *Polygonia c-album* and (ii) *Inachis io* by day in the non-diapause experiment, in which the butterflies reproduced without prior diapause, and (*b*) post-diapause adult survival of (i) *P. c-album* and (ii) *I. io* in the hibernation experiment, in which individuals had overwintered before the onset of reproduction. The pre-reproductive lifespan of *P. c-album* individuals was on average 230 days, and that of *I. io* individuals was on average 184 days. Females are represented by filled circles and solid lines, while males are shown by open circles and dashed lines. See tables 2 and 3 for the sample sizes of each category.

individuals in the hibernation experiment were surprisingly similar to the total lifespans of the individuals in the non-diapause experiment. This may appear surprising in view of the fact that the diapausing *P. c-album* individuals were on average 230 days old, and *I. io* on average 184 days old, at the start of the hibernation experiment, which means that their actual individual lifespans were correspondingly that much longer than those of the non-diapause individuals. This result suggests that pre-reproductive costs do not have a strong negative effect on post-diapause lifespan, and supports the notion that there is a coupling between lifespan and reproductive output in females, which selects for an appreciably longer female lifespan. Hence, the comparison between the two experiments brings home the message that it is the reproductive lifespan that matters, or as formulated by Indiana Jones: 'it's not the years that count, it's the mileage'.

The frequency of breeding opportunities has been suggested to explain the variation in lifespan among several species of tropical fruitflies (Sevenster & Van Alphen 1993), and a comparative study of flesh flies showed that non-diapausing species with a desynchronized phenology had longer adult lifespans than did diapausing species with synchronized development (Denlinger *et al.* 1988). Moreover, Tatar *et al.* (1997) suggested that high-elevation populations in a sibling-species complex of *Melanoplus* grasshoppers had evolved accelerated rates of ageing owing to selection on reproductive schedules, which are truncated by the early onset of winter at high altitudes.

The evolution of ageing can be seen from a vantage point of evolutionary ecology or genetics, and there is considerable consensus regarding the life-history-theory view that the evolution of the reproductive lifespan can be seen as 'a balance between selection to increase the reproductive events per lifetime and trade-offs that increase the intrinsic sources of mortality with age' (Stearns & Hoekstra 2000, p. 165). From a genetic viewpoint there are two mechanisms that could account for such an effect: the 'antagonistic pleiotropy' hypothesis of Williams (1957), and the 'disposable soma' hypothesis of Kirkwood (1987), which specifies age-specific expression of detrimental mutations. Although the two hypotheses are not mutually exclusive, the first can be characterized as a kind of trade-off in which early excellent performance is traded off against a correlated impaired performance later in life, whereas the 'disposable soma' hypothesis simply acknowledges that selection should be stronger on excellent performance early in life, and so deleterious mutations that are expressed late in life are free to accumulate without having a strong negative effect on fitness. Recently, experimental work on *Drosophila melanogaster* has reported rather strong support for the 'antagonistic pleiotropy' hypothesis, mainly because changes to select for early fecundity in lines previously selected for late fecundity rapidly result in changes in mortality, changes that are so quick that it seems unlikely that there has been time for the accumulation of deleterious mutants (Sgrò & Partridge 1999; Rose *et al.* 2002). The results reported in this paper—that

sex-specific mortality is associated with mating system, and that there is a concomitant sex difference in the importance of extended longevity—can be understood equally well in terms of the 'disposable soma' and the 'antagonistic pleiotropy' hypotheses; hence, they add support to the general life-history theory of ageing and indicate that selection can mould sex-specific responses in reproductive lifespan in accordance with life-history theory.

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