Division of labour influences the rate of ageing in weaver ant workers

Michel Chapuisat[1,2*] and Laurent Keller[2]

[1]*Institute of Ecology, Biology Building, University of Lausanne, 1015 Lausanne, Switzerland*
[2]*Department of Genetics, La Trobe University, 3083 Bundoora, Victoria, Australia*

The evolutionary theory of ageing predicts that the timing of senescence has been primarily shaped by the extrinsic mortality rate, which causes selection intensity to decline over time. One difficulty in testing the evolutionary theory of ageing is that extrinsic mortality risk is often confounded with body size and fecundity, which may also directly affect lifespan. Social insects with a pronounced division of labour between worker castes provide a unique opportunity to study the direct effect of extrinsic mortality on the evolution of ageing rates independently of body size, reproductive effort and genetic configuration. In the weaver ant, *Oecophylla smaragdina*, the major (large) workers perform the risky tasks outside the nest, while the minor (small) workers stay within the highly protected arboreal nest. Hence, this pronounced division of labour is associated with high differences in extrinsic mortality risks. The evolutionary theory of ageing predicts that the minor workers should have a longer intrinsic lifespan than the major workers. In line with this prediction, we found that in a protected environment the minor workers lived significantly longer than the major workers did. Hence, the ageing rate appears to have been moulded by variation in the extrinsic mortality rate independently of size, reproductive effort and genetic configuration.

Keywords: ageing; lifespan; *Oecophylla*

1. INTRODUCTION

Senescence constitutes an evolutionary paradox, because the intrinsic deterioration of organisms as they age limits the reproductive potential of individuals. If organisms can function well in youth then why can they not continue to do so in old age (Partridge & Barton 1993; Kirkwood & Austad 2000)? According to the evolutionary theory of ageing, all multicellular organisms with distinct parents and offspring senesce because extrinsic mortality results in a progressive decrease in the strength of selection with increasing age (Medawar 1952; Williams 1957; Hamilton 1966; Rose 1991). Under this general principle, two non-mutually exclusive evolutionary hypotheses may account for ageing. First, the antagonistic pleiotropic hypothesis proposes that genes that increase fitness early in life have detrimental effects on survival late in life (Williams 1957; Rose 1991). At the mechanistic level, there may be trade-offs between the investment of metabolic resources into repair mechanisms and into survival or reproduction. The 'disposable soma' concept combines mechanistic and evolutionary explanations by stressing that somatic maintenance is only required as long as the probability of surviving in the wild is significant (Kirkwood 1977; Kirkwood & Austad 2000). Second, the mutation accumulation hypothesis postulates that ageing is caused by late-acting deleterious mutations that have little or no effect earlier in life (Medawar 1952; Rose 1991). One major prediction of both evolutionary hypotheses is that an increase in the extrinsic mortality rate should result in the evolution of a higher ageing rate and a shorter intrinsic lifespan (Medawar 1952; Williams 1957; Hamilton 1966; Kirkwood 1977; Rose 1991; Stearns 1992; Partridge &

Barton 1993; Charlesworth 1994; Kirkwood & Austad 2000). In contrast, purely physiological theories of senescence make no predictions about the relationship between extrinsic mortality and the evolution of the ageing rate. They do frequently predict an association between the duration of life and the metabolic rate, whereby organisms with lower metabolic rates per unit of mass should have greater lifespans (Sohal 1986; Finch 1990). In animals, a larger body size is generally associated with a lower metabolic rate per unit of mass, a longer development time and a longer lifespan (Peters 1983; Stearns 1992). Thus, all else being equal, purely mechanistic theories of ageing generally predict that large animals should live longer than small animals do.

To date, attempts to test the evolutionary theory of ageing have been relatively limited (Bell 1984; Rose 1991; Zwaan 1999). In recent times, several comparative analyses (Austad & Fisher 1991; Promislow 1991; Rose 1991; Keller & Genoud 1997; Ricklefs 1998; Dudycha 2001) and experimental studies conducted mostly in *Drosophila* (Luckinbill *et al.* 1984; Rose 1984; Hillesheim & Stearns 1992; Partridge & Fowler 1992; Zwaan *et al.* 1995*a,b*; Partridge *et al.* 1999; Stearns *et al.* 2000) have provided support for the evolutionary theory of ageing. However, a major problem with many of these studies is that differences in extrinsic mortality rates are often confounded with metabolic and physiological differences that may directly influence lifespan according to purely mechanistic theories of senescence. For example, there is a strong relationship between the size of organisms and their lifespan, but it is difficult to determine whether this relationship stems from a negative association between size and extrinsic mortality or metabolism (Promislow & Harvey 1990; Gaillard *et al.* 1994). Moreover, selection experiments in the laboratory have generally influenced not only the intrinsic lifespan but other life-history traits such as

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age at reproduction and fecundity (Luckinbill *et al.* 1984; Rose 1984; Hillesheim & Stearns 1992; Partridge & Fowler 1992; Zwaan *et al.* 1995*a,b*; Partridge *et al.* 1999; Stearns *et al.* 2000). Due to the fact that reproduction itself is costly and reduces longevity in many organisms (Medawar 1952; Hamilton 1966; Williams 1966; Partridge *et al.* 1987; Promislow 1998; Westendorp & Kirkwood 1998), it is often difficult to determine whether an increase in longevity is due to a reduction in the rate of extrinsic mortality or to selection for increased late fecundity (Zwaan 1999).

Owing to their peculiar life history, highly eusocial insects with specialized worker and queen morphological castes (ants, termites and the honeybee) provide a good system for disentangling evolutionary and mechanistic ageing theories. As termite, bee and ant queens live in colonies that are sheltered and heavily defended against predators, the evolutionary theory predicts that queens of eusocial species should have a longer lifespan than solitary insects. A comparative study (Keller & Genoud 1997) indeed showed that the evolution of eusociality has been associated with a 100-fold increase in lifespan. This provides strong support for the evolutionary theory of ageing, since purely mechanistic hypotheses of senescence do not predict an association between the rate of extrinsic mortality and intrinsic lifespan. This study also demonstrated that monogyne ants (those with a single queen per colony) have longer lifespans than polygyne ants (those with multiple queens per colony). This difference again supports the evolutionary theory of ageing, because polygyne queens should suffer higher extrinsic mortality rates than monogyne queens (Keller & Genoud 1997).

The social habits of ants provide another unique way of testing the evolutionary theory of ageing. Many species of ants have castes of workers that are morphologically differentiated and specialize in different tasks. This division of labour frequently results in differences in the extrinsic mortality rate. Thus, the major (large) workers commonly ensure most of the foraging and colony defence, whereas the minor (small) workers remain within the nest where they take care of the brood (Hölldobler & Wilson 1990). In this case, the evolutionary theory predicts that the major workers should age more quickly and have shorter intrinsic lifespans, because they face higher extrinsic mortality than the minor workers do. In contrast, purely physiological theories of ageing predict a longer lifespan for the major workers due to the lower metabolic rate per unit of mass associated with their larger size (Wilson 1980; Porter & Tschinkel 1985; Porter 1986; Lighton *et al.* 1987; Lighton & Bartholomew 1988; Calabi & Porter 1989). The comparative study of lifespans between worker castes also presents the unique and interesting property of not being confounded with possible differences in the cost and timing of reproduction. This is because workers typically do not reproduce in the presence of the queen (Bourke 1988; Hölldobler & Wilson 1990) and thus, there is no trade-off between the energy invested into direct reproduction and into other functions such as repair mechanisms enhancing longevity. However, workers maximize their inclusive fitness by increasing the reproduction of the queen, and they are selected for maximizing colony productivity. Therefore, worker lifespan can still be moulded by extrinsic mortality risk independently of direct reproduction, e.g. if there are trade-offs between the resources invested into individual somatic maintenance and into functions increasing worker or colony performances.

To test whether differences in extrinsic mortality have resulted in the evolution of a variable intrinsic lifespan of the workers, we selected the ant *Oecophylla smaragdina*. Two characteristics make this species particularly suited for such studies. First, weaver ants of the genus *Oecophylla* exhibit a clearly bimodal size distribution, with almost no overlap between the size of the minor and major workers (Wilson 1953; Hölldobler & Wilson 1990). Second, the size dimorphism in *Oecophylla* is associated with a pronounced division of labour (Weber 1949; Hölldobler & Wilson 1990). In *O. longinoda* and *O. smaragdina*, the minors stay within the nests, where they care for the brood and tend scale insects that produce honeydew. In contrast, the major workers do the foraging, territorial defence, nest guarding and repairs (Weber 1949; Hölldobler & Wilson 1990; E. O. Wilson, personal communication). Colony defence and foraging are dangerous tasks associated with high mortality in ants, and this is likely to be particularly true in *Oecophylla* where majors aggressively defend extensive territories against intra- and inter-specific competitors (Hölldobler & Wilson 1977; Hölldobler & Lumsden 1980). The mortality rates of the minor and major workers have not been measured in the field. However, because of the marked division of labour, it is very likely that the major workers suffer from much higher rates of extrinsic mortality than the minor workers do. Hence, the worker caste system in the genus *Oecophylla* is well suited for testing whether intrinsic lifespan has been moulded by variation in extrinsic rates of mortality independently of the cost and timing of reproduction.

2. MATERIAL AND METHODS

Three colonies of *O. smaragdina* were collected on 21 September 1998 in Townsville, Queensland, Australia, and shipped by plane to La Trobe University, Victoria, Australia. Ants were kept for a few days in big containers until small experimental colony fragments were set up.

Experimental colony fragments were established in 3.75 l plastic containers (21 cm × 21 cm × 7 cm) side-coated with fluon and covered with a mesh lid to prevent escape. Each unit was given a nest (Passera *et al.* 1988) covered with a dark plastic sheet that could be removed for inspection. Experimental groups were kept at 22–25 °C, under a 12 L : 12 D cycle and fed *ad libitum* with an identical diet of water, diluted honey and freshly killed insects. Under these experimental conditions, both the major and minor workers performed only limited foraging and spent most of the time being inactive within the nest.

At the beginning of the experiment, the ratio of major to minor workers was 1.2, 1.4 and 2 for the three colonies, respectively. This is close to the ratio found in wild colonies (Lokkers 1990; C. Lokkers, personal communication). Most major workers selected at the start of the experiment were very young (callow) workers, recognizable by their pale colour and soft cuticles. The colonies collected contained only a few callow minor workers, presumably because the callow stage is shorter for small workers. Therefore, we had to use mostly adult minor workers and subsequently most of the minor workers were of unknown age, but older than the major workers at the start of the experi-
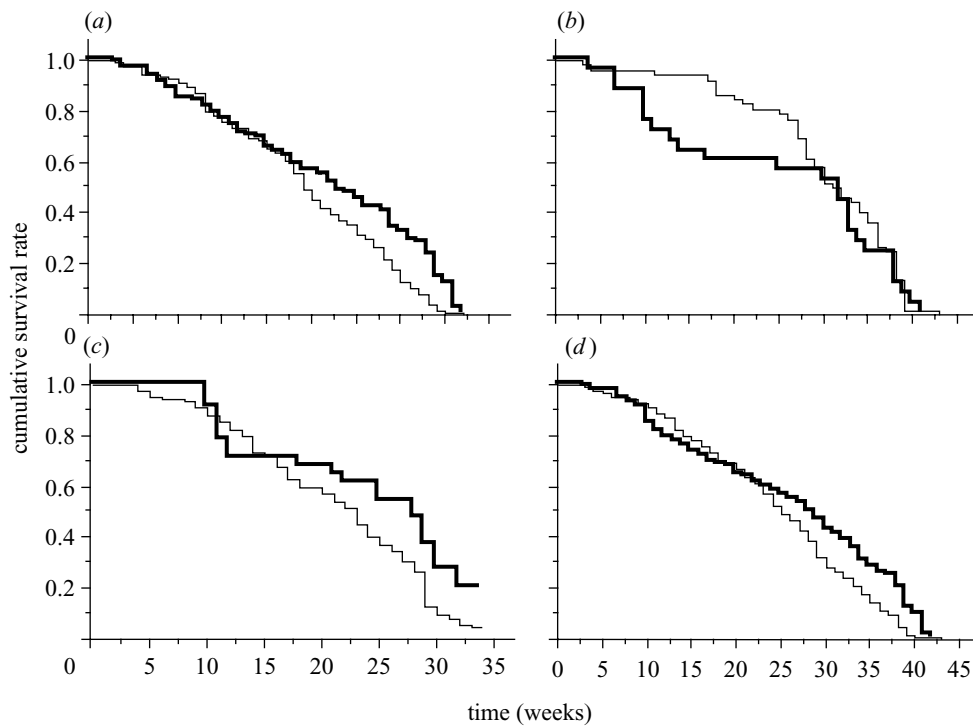
Figure 1. Kaplan–Meier cumulative survival rates of the minor (thick line) and major (thin line) workers for the three colonies and overall. (*a*) Colony 1, $N = 131$, $n = 98$; (*b*) colony 2, $N = 52$, $n = 26$; (*c*) colony 3, $N = 90$, $n = 35$; and (*d*) all colonies, $N = 273$, $n = 159$. N and n give the number of major and minor workers included in the analysis.

ment. This makes our comparison highly conservative, because the hypothesis is that minors should live longer than majors.

The containers and nests were carefully inspected once a week. All corpses were removed and the head widths measured. The experiment lasted until the death of the last individual, which occurred at week 43. Individuals that died during the initial two weeks after the start of the experiment were removed from the analysis on the assumption that their death was due to the effect of collection, transport and installation in the experimental colonies, rather than natural ageing. The numbers of major and minor workers remaining after this initial period of acclimatization are given in the legend to figure 1. After the first two weeks, *ca.* 5% of the workers died accidentally. In particular, Argentine ants (*Linepithema humile*) invaded one experimental colony fragment and killed all *Oecophylla* in week 34. These cases of accidental death were right censored in the survival analysis. The product-limit survival estimates (Kaplan & Meier 1958) of the minor and major workers were computed, and differences in the survival functions were tested with a Mantel–Cox logrank test, as implemented in the computer program STATVIEW 4.5 (Abacus Concepts, Berkeley, CA).

3. RESULTS

The worker size was clearly bimodal in our sample. The major workers were *ca.* 8.0 mm long with a head width (across the eyes) of 1.5 mm. The minor workers were *ca.* 4.5 mm long with a head width (across the eyes) of 1.0 mm. Workers with a head width above or below 1.25 mm were classified as majors or minors, respectively. The dry weight of the major workers was about three times that of the minor workers (mean ± s.d. = 3.0 ± 1.0 mg for the majors, $n = 10$, and 1.0 ± 0.1 mg for the minors, $n = 10$; Mann–Whitney U-test, $Z = -3.8$, $p < 0.001$).

All workers died within 43 weeks (figure 1). Overall, the survival functions of the minor and major workers were significantly different (Mantel–Cox logrank test, stratified by colony, $\chi^2 = 12.9$, d.f. = 1, $p < 0.001$), with the minor workers having a higher probability of reaching older age (figure 1). There was a tendency of a lower survival probability for the minor workers between weeks 10 and 20. This pattern can be explained by the fact that the minor workers were of variable age at the beginning of the experiment (see § 2), whereas all the majors were callow (young) workers.

Similar results were obtained on analysing data at the colony level. The survival functions were significantly different in two out of the three colonies (Mantel–Cox logrank test, $\chi^2 = 12.1$, d.f. = 1, $p < 0.001$ and $\chi^2 = 5.8$, d.f. = 1, $p < 0.02$ for colonies 1 and 3, respectively), with the minor workers tending to live longer than the major workers (figure 1). No significant difference in survival was found in the smaller colony fragment (colony 2; Mantel-Cox logrank test, $\chi^2 = 0.5$, d.f. = 1, $p = 0.49$), possibly due to the lower power of the test in this colony.

4. DISCUSSION

Our study demonstrates that, as predicted by the evolutionary theory of ageing, differences in extrinsic mortality are strongly associated with intrinsic mortality rates (hence lifespan) in the ant *O. smaragdina*. In captivity, the minor workers had a significantly higher survival probability than the major workers, despite the fact that the majors have a threefold larger body mass. Our study does not allow a precise determination of the difference in the lifespan between the minor and major workers, because the age of the minors varied at the beginning of the experiment,

whereas the majors were all young callow individuals. Thus, our comparative study was conservative and only allowed us to conclude that the minors have a higher survival probability than the majors. This demonstrates that lifespan is not strongly constrained by the size of an organism. Smaller organisms can have a longer lifespan than larger ones when they suffer from a lower extrinsic mortality rate.

The finding that the minors had a longer lifespan despite their smaller size strongly supports a direct effect of extrinsic mortality on the evolution of the rate of ageing, because, everything else being equal, a bigger size and presumably a lower metabolic rate per unit of mass should be associated with a longer lifespan. In ants, as in most other taxa, the longevity of the workers is also size related, with larger species generally living longer than smaller ones (Porter & Tschinkel 1985). At the intraspecific level, data are available for a single species, the fire ant, *Solenopsis invicta*. In this species workers vary in size, but in contrast to *O. smaragdina* the difference in size is not associated with a clear difference in extrinsic mortality risk. The larger workers tend to specialize on tasks such as nest construction or large prey retrieval, whereas the minor workers do most of the brood care (Wilson 1978; Mirenda & Vinson 1981). However, the major workers spend most of their time being inactive (Mirenda & Vinson 1981), and the minor workers frequently forage and work outside the nest (Wilson 1978; Mirenda & Vinson 1981). Hence, the extrinsic mortality risk of minor and major workers is probably more balanced in *S. invicta* than in *O. smaragdina*. Interestingly, under laboratory conditions larger *S. invicta* workers live from 50 to 140% longer than smaller workers (Porter & Tschinkel 1985; Calabi & Porter 1989). The larger workers also have a lower metabolic rate per unit of mass than the smaller workers (Porter & Tschinkel 1985; Calabi & Porter 1989), indicating that, in the absence of a marked difference in extrinsic mortality rates, lifespan might indeed correlate with body size and metabolic rate.

Another interesting aspect of the comparison of ageing rates between castes in social insect colonies is that individuals that differ in intrinsic longevity usually share the same configuration of genes, as they generally derive from the same parents. Hence, differences in rates of ageing between castes should mostly be due to differential patterns of gene expression that are initially triggered by environmental factors, rather than to fixed genetic differences (Hölldobler & Wilson 1990; Bourke & Franks 1995). Interestingly, the accumulation of late-acting deleterious mutations can only occur at loci that are expressed late in life in castes that have shorter lifespans, but are inactive at a similar age in castes that have longer lifespans. At other loci, late-acting deleterious mutations should be eliminated from the germ-line by selection on the caste that has a longer intrinsic lifespan. Hence, the role of late-acting deleterious mutations in causing differential ageing among castes will be limited by the number of genes that are expressed exclusively in the short-lived caste of adults. In contrast, the 'disposable soma' concept fully applies to sterile individuals living in colonies. Ageing rates in workers may depend on trades-offs between somatic maintenance and other functions that increase individual or colony performances. Ultimately, investment

into somatic maintenance may decrease as the extrinsic mortality risk increases, and caste-specific expression of genes with antagonistic pleiotropic effects may have been selected if this increased colony productivity.

In conclusion, the present study offers a novel comparative analysis of lifespan data within a system where individuals differ in extrinsic mortality risks, but share the same configuration of genes. Our study demonstrates that minor workers have a longer lifespan than major workers, despite their smaller size and presumably higher metabolic rate per unit of mass. Thus, lifespan seems to be less affected by physiological constraints associated with differences in body size and metabolism than by differences in the extrinsic mortality rate. Moreover, this study shows that changes in extrinsic mortality risks can affect the evolution of ageing rates independently of indirect effects associated with the timing of reproduction and fecundity. This association between extrinsic mortality risk and ageing rate might be due to trade-offs between the amount of resources invested into somatic maintenance and into other functions that increase individual performance and colony success.

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