



Lifespan of a *Ceratitis* fruit fly increases with higher altitude

PIERRE-FRANÇOIS DUYCK^{1,2*}, NIKOS A. KOULOSSIS³, NIKOS T. PAPADOPOULOS⁴, SERGE QUILICI², JANE-LING WANG⁵, CI-REN JIANG⁶, HANS-GEORG MÜLLER⁵ and JAMES R. CAREY⁷

¹CIRAD, UPR 26, PRAM – BP 214, 97285 Lamentin, Cedex 2, Martinique, French West Indies, France

²CIRAD 3P, UMR PVBMT Cirad/Université de La Réunion, Pôle de Protection des Plantes, F-97410 St Pierre, La Réunion, France

³Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

⁴Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, 384 36 Nea Ionia, Volos, Greece

⁵Department of Statistics, University of California, One Shields Avenue, Davis, CA 95616, USA

⁶Department of Statistics, University of California, Berkeley, CA, 94720, USA

⁷Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616, USA

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Variation in lifespan may be linked to geographic factors. Although latitudinal variation in lifespan has been studied for a number of species, altitude variation has received much less attention, particularly in insects. We measured the lifespan of different populations of the Natal fruit fly, *Ceratitis rosa*, along an altitudinal cline. For the different populations, we first measured the residual longevity of wild flies by captive cohort approach and compared the F_1 generation from the same populations. We showed an increase in lifespan with higher altitude for a part of the data obtained. For the field-collected flies (F_0) the average remaining lifespan increased monotonically with altitude for males but not for females. For the F_1 generation, the longevity of both males and females of the highest-altitude population was longer than for the two other lower-altitude populations. This relationship between altitude and lifespan may be explained by the effects of temperature on reproduction. Reproductive schedules in insects are linked to temperature: lower temperature, which is characteristic of high-altitude sites, generally slows down reproduction. Because of a strong trade-off between reproduction and longevity, we therefore observed a longer lifespan for the high-altitude populations. Other hypotheses, such as different predation rates in the different sites, are also discussed. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **101**, 345–350.

ADDITIONAL KEYWORDS: biodemographic studies, *Ceratitis rosa*, longevity, Natal, tephritidae.

INTRODUCTION

Variation in longevity in the wild may be caused by different external factors. Classical theories on the evolution of senescence predict that populations that have experienced external high mortality rates

will senesce more quickly (Williams *et al.*, 2006). For example, populations that have been subjected to high predation rate have evolved to a shorter lifespan (Reznick, Bryant & Holmes, 2006).

Variation in longevity may also be linked to geographic abiotic factors. Longevity in the wild may vary with latitude and altitude, in relation to temperature. Most studies on the relationship between senescence and geographic variation have

*Corresponding author. E-mail: duyck@cirad.fr

been carried out in birds. Different studies have shown that the senescence rate among bird species increases with latitude, as expected because of slow life-histories at low latitudes (Fogden, 1972; Møller, 2007; Wiersma *et al.*, 2007). The shorter lifespan in birds from temperate compared to tropical climates may be explained by birds having evolved a high basal metabolic rate linked to a high thermogenic capacity (Wiersma *et al.*, 2007). However, in ectotherms, such as insects, lifespan usually increases with latitude, and this variation of lifespan within a species has been recently explained by the metabolic theory of ecology (Munch & Salinas, 2009).

Although latitudinal variation in lifespan has been studied for a number of species, variation with altitude, in particular in insects, has received much less attention, most likely because altitudinal clines involve shorter distances, with gene flow between populations probably being important (Karl, Janowitz & Fischer, 2008). In a study on grasshoppers in California, Tatar, Gray & Carey (1997) showed a decrease in longevity with high altitude. It was hypothesized that high elevation populations have evolved an accelerated senescence as a result of selection on reproductive schedules, which are potentially structured by severe winter conditions at the elevated sites. However reproductive schedules in insects are linked to temperature and lower temperature at high elevation sites should slow down reproduction. Experimentally increased reproductive activity is generally associated with a decreased lifespan (Williams, 1966; Gustafsson & Part, 1990; Chapman *et al.*, 1998). Because of this strong trade-off between reproduction and longevity, a longer lifespan should be observed in high elevation places. In a site where winter conditions are not strong enough to stop reproduction, we predict an increase in lifespan with higher altitude.

We tested this prediction along an altitudinal gradient in a tropical area (La Réunion, an island in the south-western Indian Ocean) using a *Ceratitis* fruit fly species. *Ceratitis rosa* is very closely genetically and ecologically related to the medfly, *Ceratitis capitata* (Malacrida *et al.*, 1996; Torti *et al.*, 1998; Baliraine *et al.*, 2004; De Meyer *et al.*, 2008), which is an important model species for ageing studies (Carey, 2001). *Ceratitis rosa* is present in Eastern and Southern Africa as well as in some Indian ocean islands (De Meyer *et al.*, 2008). It invaded La Réunion in the mid-20th century and is now present in most parts of the island (Duyck, David & Quilici, 2006). *Ceratitis rosa* is able to develop in a wide range of temperatures and is therefore found from sea level up to an altitude of 1500 m. Pupae of this species survive better in humid compared to dry conditions (Duyck *et al.*, 2006). As for other fruit fly species, the development and reproductive ability of *C. rosa* are linked

to temperature, with lower temperature causing decreased ovarian maturation rates and therefore decreased fecundity (Duyck & Quilici, 2002).

The present study aimed to test the prediction that the lifespan of *C. rosa* increases with altitude. We measured the lifespan of different populations of *C. rosa* along an altitudinal cline. For the different populations, we first measured the residual longevity of wild flies by the captive cohort approach (Carey *et al.*, 2008). Because differences in residual longevity may imply different longevities, a different age structure, or both, we measured lifespan and compared the F_1 generation from the same populations.

MATERIAL AND METHODS

COLLECTION SITES

We collected males and females of *C. rosa* from three sites along a transect of altitude (300, 600, and 900 m) in La Réunion island (Indian Ocean, 21°20'S, 55°15'E) in December 2007 (Table 1). Each collection site consisted of a Creole garden with many fruit species and no insecticide applications. The seasonal characteristics of the sites varies systematically from low to high elevation, with decreasing temperature and increasing rainfall with increasing altitude (Table 1).

LIFESPAN MEASUREMENT

In each site, males and females fruit flies were collected for one day using 20 'Tephri-traps' (Sorygar) with '3-lures' (Suterra), a nitrogen-based food that is attractive for both sexes of various *Ceratitis* spp., but is more effective for females than males (Epsky *et al.*, 1999). These live-caught flies were then transported to the laboratory. For each site, 92 flies (46 males and 46 females) were placed in individual containers for mortality measurement, whereas the remaining flies ($N = 100-200$) were placed together in a cage (30 × 30 × 30 cm) to construct the F_1 cohort. In each cage, an egg laying device with spaced holes containing a piece of *Citrus* was used to collect eggs. Larvae were reared on an artificial diet (Duyck & Quilici, 2002). After adult emergence from pupae, 92 flies (46

Table 1. Climatic parameters at the three study sites

	Altitude (m)		
	300	600	900
Mean annual temperature (°C)	21.9	19.5	18.0
Minimum temperature (°C)	11.2	8.7	6.2
Maximum temperature (°C)	33.4	31.5	29.6
Mean annual rainfall (mm)	1100	1400	1700

males and 46 females) were placed in individual cages for mortality measurement. In total ($F_0 + F_1$), 552 flies were observed individually.

The experimental procedure was conducted in accordance with standard methods for the study of Tephritidae longevity (Carey, 2003). The present study was conducted in the laboratory under a 12 : 12 h light/dark cycle at 25 ± 1 °C and $65 \pm 10\%$ relative humidity. Light was provided by daylight tubes. Individual cages consisted of a transparent plastic cup (height 12 cm, base diameter 5 cm, top diameter 7.5 cm), which was placed upside down, and glued to the lid of a plastic Petri dish (diameter 9 cm) with adult food [a mixture of yeast hydrolyzate (ICN Biochemicals) and sugar, 1 : 4] and water. Adult food was supplied *ad libitum* on the floor of the cage, and water was provided by a cotton wick that passed through a small hole in the Petri dish lid to an underlying Petri dish base, which was filled with water. A lateral window covered with mesh was perforated on the cup's side for ventilation. Mortality in each individual cage was recorded daily.

CALCULATIONS AND STATISTICAL ANALYSIS

Life expectancy at birth was calculated as $\sum_{x=0}^{\infty} \frac{1}{l_x}$ where l_x is the fraction of the cohort alive at age x (Carey, 2001). Confidence intervals for life expectancy were estimated as the 2.5th and 97.5th percentiles of a bootstrap distribution resampled 1000 times (Efron & Tibshirani, 1993; Caswell, 2001).

After verification of normality, data longevities of the F_0 and F_1 generations were subjected to a two-way analysis of variance (ANOVA) that included the effects of the sex (males, females), altitude (300, 600, and 900 m), and interactions between the two factors.

RESULTS

For the F_0 generation, ANOVA showed a significant effect of altitude and of interaction between sex and altitude, whereas no effect of sex on longevity was observed (Table 2). To further understand the effect of altitude on longevity, we compared the model with sex only to the complete model with sex, altitude, and interaction. This gave a global effect of altitude and sex \times altitude on longevity, which was highly significant ($F_{4,270} = 3.54$, $P = 0.008$).

For the F_1 generation, ANOVA showed a strong effect of altitude, whereas no effect of sex or of sex \times altitude was observed on longevity (Table 2).

Differences in life expectancies among populations were substantial, in the range 116–165 days for males and 106–173 days for females (Table 3). The life expectancy of F_0 males showed a strong increase with increasing altitude, whereas no differences in the life expectancy of F_0 females were observed. For F_1 males and females, a higher life expectancy was observed at 900 m compared to 300 and 600 m.

DISCUSSION

Our prediction of increase in lifespan with higher altitude is partly verified in the present study. For field collected flies (F_0), the average remaining lifespan of males monotonically increased with altitude, whereas the remaining lifespan of females was not influenced by the collection place. For the F_1 generation, the longevity of both males and females of the highest-altitude (900 m) population was longer than for the two other lower-altitude populations (300 and 600 m). Because distances among sites are relatively low, gene flow among *C. rosa* populations may occur (Baliraine *et al.*, 2004). However, differences in longevity among the F_1 populations are assumed to

Table 2. Analysis of variance tables for the captive cohort F_0 (a) and the F_1 generation. Significant effects ($P < 0.05$) are indicated in bold

Source of variance	d.f.	Sum of squares	Mean square	<i>F</i>	<i>P</i>
Captive cohort F_0					
Sex	1	3 107	3 107	0.79	0.373
Altitude	2	30 221	15 111	3.87	0.022
Sex \times Altitude	2	25 196	12 598	3.22	0.041
Residuals	270	1 055 246			
F_1 generation					
Sex	1	3 087	3 087	0.38	0.5386
Altitude	2	170 589	85 294	10.48	< 0.0001
Sex \times Altitude	2	12 158	6 079	0.75	0.4749
Residuals	270	2 198 096	8 141		

Table 3. Adult life expectancy (at capture for F_0 , at birth for F_1) (days)

Generation	Sex	Altitude (m)					
		300	600	900			
F_0	Male	116	(101, 129)	141	(123, 159)	165	(147, 184)
	Female	146	(128, 162)	147	(128, 165)	148	(129, 168)
F_1	Male	130	(105, 154)	117	(90, 143)	165	(141, 188)
	Female	106	(81, 130)	113	(85, 145)	173	(147, 201)

Confidence intervals were estimated as the 2.5th and 97.5th percentiles of a bootstrap distribution resampled 1000 times (Efron & Tibshirani, 1993; Caswell, 2001).

have a genetic component. This relationship between altitude and lifespan may be explained by the effect of temperature on reproduction. Reproductive schedules in insects are linked to temperature: lower temperature, which is characteristic of high-altitude sites, generally slows down reproduction. This is the case for *C. rosa*, which show decreasing ovarian maturation rates, and therefore fecundity, with lower altitude (Duyck & Quilici, 2002). Because of the strong trade-off between reproduction and longevity, we therefore observed a longer lifespan for the high-altitude populations. Early in the 20th Century, Ripley & Hepburn (1930) observed that, at high altitudes in South Africa, the Natal fruit fly, was overwintering at the adult stage.

Other hypotheses could also explain such a pattern. For example, different predation rates may explain variation in senescence rates (Reznick *et al.*, 2004; Reznick *et al.*, 2006). Populations subjected to a lower predation rate may evolve a longer lifespan. This means that, in our case study, populations from the high-altitude site would have been exposed to lower predation rates. Although data are lacking on adult *C. rosa* natural enemies, such as ants, spiders or wasps, it is likely that they might be more abundant in warm low altitude areas. In addition, larval host-fruits and adult food have been shown to have effects on the life-history traits of Tephritidae, including longevity and fecundity (Brévault, Duyck & Quilici, 2008; Duyck *et al.*, 2008). Because different altitudes result in different habitats with probably different host species and adult food sources, the differential longevities among sites may be also partly explained by these factors. These different hypotheses (i.e. temperature, natural enemies, and resources) may be combined to explain the observed effects of altitude.

Few studies have examined the effect of altitude on longevity in insect species. By contrast to the conclusions of the present study, Tatar *et al.* (1997) showed an inverse pattern of decreasing longevity with increasing altitude in *Melanoplus* grasshoppers. However, their findings may be a result of the

species studied as well as the location where these species have evolved, leading to the requirement of accelerated reproductive schedules during the summer because of the severe winter conditions at the elevated sites. The results of the present study suggesting an increased longevity with altitude are in accordance with those obtained by Karl & Fischer (2009). Comparing populations of the butterfly *Lycaena tityrus* (Lycaenidae) from low and high altitudes, these authors showed an increased lifespan with higher altitude when insects were fed on a full diet. This trend of greater longevity in high-altitude populations has also been shown for *Drosophila buzzatii* Patterson & Wheeler (1942) (Norry *et al.*, 2006) and for some amphibians (Morrison & Hero, 2003; Morrison, Hero & Browning, 2004).

By contrast to the conclusions of the present study, investigations in birds from a tropical warm environment have reported a longer lifespan compared to birds from a temperate cold environment (Fogden, 1972; Møller, 2007; Wiersma *et al.*, 2007). This may be explained by the fact that, contrary to insects, birds are homeothermic organisms. In cold environments, birds likely have evolved metabolic machinery with a high thermogenic capacity, which may mandate a high basal metabolic rate (Wiersma *et al.*, 2007). Møller (2007) showed that the senescence rate among bird species increases with increasing latitude, and therefore with decreasing temperature. This relationship in birds is explained by the hypothesis that tropical birds have evolved life-history traits indicative of a slow rate of ageing linked to a reduced basal metabolic rate (Wiersma *et al.*, 2007).

Surprisingly, for some of the cohorts, life expectancy was longer for the F_0 generation than the mean captive lifespan of the F_1 generation. A possible interpretation for this might be that adults trapped in the field (F_0) were generally young and their early experience in the field was beneficial and increased their longevity. For example adults in the field may have found some nutrients that we did not provide under laboratory conditions. This is in accordance with the findings of

Carey *et al.* (2008) who showed that the lifespans of a fraction of once-wild flies would exceed the lifespans of any never-wild flies. It was hypothesized that there is a window in the early adulthood of the medfly when the presence of certain amounts or types of bacteria in the diet is important and that wild flies have access to these bacteria, whereas reference flies in the laboratory do not, as shown for *Drosophila* (Brummel *et al.*, 2004). Another possible explanation relates to demographic selection arguments (Vaupel & Carey, 1993). Field flies may have died easily during a certain period in their early life and most of the captured flies had already passed that period. Therefore, captured F_0 flies could represent a selected cohort for which all short-lived flies have already died before the beginning of the experiment. However, this selection argument would change the frequency of the older flies but not their absolute lifespans – see Carey *et al.* (2008) for a complete discussion on this point.

A significant interaction between sex and altitude on longevity has been shown for the F_0 generation because there is no effect of altitude on females, whereas increasing longevity is observed with increasing altitude in males. Differences between the F_0 and F_1 generations may reflect differences in longevity, in age structure, or both. The data obtained in the present study suggest that the age structure in the trap (F_0) is modified compared to a population at equilibrium. For example, Kouloussis *et al.* (2009) showed that the age structure of fruit flies may be modified by both season and trapping method. The unchanged longevity for the F_0 females might be explained by the response of females to the ‘3-lures’ attractant. In other Tephritidae species, the response of females to various attractants was shown to depend on their physiological stage (Kendra *et al.*, 2005; Rousse *et al.*, 2005). Although the different female populations originated from different habitats, their average physiological stage would be similar, and therefore their residual lifespan under laboratory conditions was not different.

Very few studies are available on the effect of altitude on longevity. Our prediction of an increase in lifespan with higher altitude has been partly verified in the present study and could invoke a temperature trade-off with reproduction. The effects of altitude variation in lifespan not only have theoretical implications for the understanding of lifespan evolution, but also have practical implications regarding the possible adaptation of ectotherms to climate warming (Tewksbury, Huey & Deutsch, 2008).

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