

## Population variability in the beetle *Timarcha maritima* (Coleoptera, Chrysomelidae)\*

Frédéric THOMAS\*\*, Thierry DE MEEUS

Centre d'Etude sur le Polymorphisme des Micro-Organismes, CEPM/UMR CNRS-IRD 9926, Equipe: "Evolution des Systèmes Symbiotiques", IRD, 911 Avenue Agropolis, B. P. 5045, 34032 Montpellier Cedex 1, France

**Abstract** We investigated variability between populations of the beetle *Timarcha maritima* over its geographic range by comparing several variables (size of individuals, fecundity, parasite load, sex ratio, pattern of pairing) in 10 populations along the French Atlantic coast. Populations exhibited significant differences for all variables considered. Our findings also indicate a major influence of the protozoan parasite *Gregarina munieri* on *T. maritima* ecology. Beetles paired assortatively according to the parasitic load, a pattern of pairing detected 3 years ago in a highly parasitized population. Size assortative pairing was never significant, even in parasite-free populations. The level of infection by gregarines was also a strong predictor of mean fecundity of females at the inter-population level. Conversely, the role of the external parasite *Pseudamansia chrysomelinus* (Acari: Canestriniidae) was weak whatever the population considered. This study underlines the need to consider parasites when studying population ecology [*Acta Zoologica Sinica* 52 (3): 491–497, 2006].

**Key words** Assortative pairing, Chrysomelidae, *Gregarina munieri*, Population ecology, *Pseudamansia chrysomelinus*, *Timarcha maritima*

## 一种叶甲 (*Timarcha maritima*) (鞘翅目: 叶甲科) 的种群变异性\*

Frédéric THOMAS\*\*, Thierry DE MEEUS

Centre d'Etude sur le Polymorphisme des Micro-Organismes, CEPM/UMR CNRS-IRD 9926, Equipe: "Evolution des Systèmes Symbiotiques", IRD, 911 Avenue Agropolis, B. P. 5045, 34032 Montpellier Cedex 1, France

**摘要** 本文研究了一种叶甲 (*Timarcha maritima*) 的种群变异性, 在此物种的整个分布范围内, 沿法国大西洋海岸选取 10 个种群, 测定个体大小、生育力、寄生量、性比、交配模式几个变量, 各变量在不同种群间呈现显著差异。我们的调查结果也表明: 原生生物 (簇虫, *Gregarina munieri*) 的寄生是影响 *T. maritima* 生态习性的主要因素; 这种甲虫的交配模式与寄生量有关, 这种交配模式 3 年前已在一个高寄生种群内发现; 个体大小与交配的关联不显著, 即便是在没有寄生的种群中也是这样; 在不同种群间, 寄生感染程度也是一个有力的指示量, 可以预示雌性个体的生育力变化; 相反, 不管观察什么种群, 外寄生种 (蜱螨, *Pseudamansia chrysomelinus*) (Acari: Canestriniidae) 的作用很小。这项研究表明: 在研究种群生态学时, 需要同时观察寄生物的影响 [*动物学报* 52 (3): 491–497, 2006]。

**关键词** 相配配对 叶甲科 *Gregarina munieri* 种群生态学 *Pseudamansia chrysomelinus* *Timarcha maritima*

An important aspect of recent theoretical and experimental studies on population ecology and adaptive processes is the recognition that these phenomena have to be examined in a metapopulation context (e. g., Hanski, 2002). The majority of plant and

animal species indeed exhibit over their entire geographic range a metapopulation structure, occupying habitats that are fragmented and heterogeneous in space and/or through time (Foster and Endler, 1999). The combination of local interactions and dis-

Received Nov. 16, 2005; accepted Feb. 08, 2006

\* This research was funded by the grants from the IRD and the CNRS

\*\* Corresponding author. E-mail: fthomas@mpl.ird.fr

© 2006 动物学报 *Acta Zoologica Sinica*

persal at various scales leads to a range of important implications for the ecology and evolution of populations. When habitat boundaries are distinct and gene flow between populations is effectively absent, populations can theoretically respond rapidly to local differences in selective regime (Endler, 1977, 1986). For numerous species, evidence is now accumulating about the way in which life history traits and behaviour indeed vary among natural populations in response to selective pressures exerted by the local environment (e.g., Lafferty, 1993; Bell and Foster, 1994; Berthold, 1993, 1999; Hewit and Butlin, 1997; Foster and Endler, 1999; Verrell, 1999; Borries et al., 2001; Guégan et al., 2001; Hamilton and Poulin, 2001; Johnson, 2001; Thomas et al., 2001). Such studies provide interesting means of identifying the selective pressures experienced by populations and hence, possible causes of adaptive differentiation in wild populations.

*Timarcha maritima* (Chrysomelidae) is a black beetle living on the French Atlantic coast (Chevin, 1992; Jolivet, 1997), is confined to dunes and is wingless. Therefore, the numerous natural and artificial interruptions occurring along the Atlantic dunes isolate several populations, between which gene flow is limited or absent. In a previous study, Thomas et al. (1999) investigated the biology of *T. maritima* in a population severely parasitized by the protozoan gut parasite *Gregarina munieri*, Schneider 1876 (Sporozoa). Hosts become infected by ingesting spores that later develop into trophozoites, a feeding stage that attaches to the intestine for a period of several days to weeks (Zuk, 1987a). Gregarines alter host nutrition because they act as a physiological barrier between the midgut cells and digested food (Harry, 1970; Zuk, 1987a, 1987b). In this population of *T. maritima*, fecundity in females and mobility in males were strongly reduced by parasitic load (Thomas et al., 1999). In addition, there was an assortative pairing with respect to parasite load (i.e., males and females were matched for parasite load), resulting presumably from parasitized females being less attractive and parasitized males less competitive (Thomas et al., 1999). Although arthropods are frequently matched for size (see Crespi 1989 for a review), this pattern of pairing was not observed in this population.

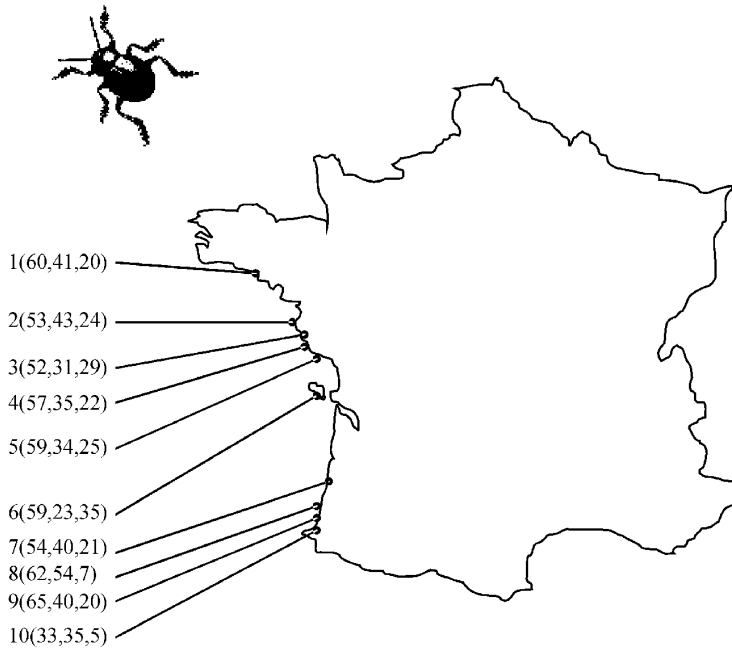
The aim of the present study was to assess the natural variability of *T. maritima* populations over its geographic range. We compared several variables (size of individuals, fecundity, parasitic load, sex ratio, pattern of pairing) from *T. maritima* collected in 10 locations along the French Atlantic coast. In addition, to provide global data on *T. maritima* biology, the second aim of this study was to explore the

ecological relevance of gregarines on the biology of this beetle, addressing the following questions (i) is the original pattern of assortative pairing for parasitic load previously found constant over time? (ii) Are paired individuals from populations free of parasites matched for size? (iii) To what extent do gregarines influence fecundity variation between populations? We also analysed the effect of these parameters on an external mite (*Pseudamansia chrysomelina* (Koch); Santiago-Blay and Fain 1994) to check if the trends observed in the internal gregarines could be generalised to any beetle associate, and for the possible interaction between these two species.

## 1 Material and methods

All beetles ( $n = 1\,692$ ) were collected during April 1999 along the dunes of the French Atlantic coast (see Fig. 1). In each site, we searched a wide section of dune for beetles in pairs and unpaired individuals. Because all sites were visited within only two weeks, we did not expect significant temporal effect. Similarly, because paired beetles usually stay from several hours to several days together, there is no reason to expect a role for time of the sampling period. All insects were preserved in alcohol (70%). In the laboratory, individuals were sexed, measured (elytron length) and dissected to estimate their gregarine load. Because the number of gregarine trophozoites per individual could be high, we estimated parasite load using the same procedure as Thomas et al. (1999): entire gut contents were placed in a Petri dish with alcohol (70%) and manually stirred for 15 s during which the distribution of trophozoites became homogeneous in the Petri dish. By placing a grid (5 mm  $\times$  5 mm) under the Petri dish, we estimated parasite load as the mean number of trophozoites per square (estimated from five squares under a dissecting microscope 400 $\times$ ) and then multiplied by the total number of squares. The measurement error in trophozoites estimates based on single squares is negligible with this procedure (see Thomas et al., 1999). However, statistical analyses were made using the raw data (i.e., counts made on these five squares). Fecundity was estimated by counting the number of yolky eggs contained in the abdomen of females. Mite load was determined by counting the number of individuals under elytra. We verified that insects did not lose some of their ectoparasites by examining the tubes.

All statistical tests were performed with the statistical software S-Plus 2000 Professional release 3. Analysis of variance was used for Gaussian data and Generalised Linear Modelling (GLiM) for Poisson or binomial data. Because dispersion of data may not fit to theoretical distributions, we used the quasi-likeli-



**Fig. 1** Localities of the different populations sampled

1: Quiberon; 2: Bretignole; 3: Gachere; 4: Aubraie; 5: Terriere; 6: Oleron; 7: Vieux Boucau; 8: Hossegor; 9: Capbreton; 10: Boucau. Numbers respectively indicate the number of pairs, of unpaired males and of unpaired females collected.

hood method with the appropriate link and variance functions as advised by the S-PLUS 2000 Guide to Statistics, Volume 1. For each regression, the minimal model was obtained with the “step” function (both backward and forward) that uses the AIC (Akaike Information Criterion) statistics of the model as a criterion (the lower the better) to keep or remove a term from the model. The significance of the terms remaining in the minimal model were then tested with a *F* test (ANOVA). The different terms were Size (the size of individual beetles), Site (from 1 to 10), Sex (the sex of the beetle), Gregarines (the abundance of gregarines in individual beetles), Acariens (the abundance of acariens on individual beetles), Fecundity (the number of eggs found in female beetles) and Paired (the paired or unpaired status, a Boolean term that was set True for paired individuals).

The different initial models were the following, with indication of the family model used (: for interaction):

Size  $\sim$  Site + Sex + Constant, Gaussian;  
 Gregarines  $\sim$  Site + Sex + Size + Sex: Size + Constant, Quasi (link = Log, variance =  $\mu$ );  
 Acariens  $\sim$  Site + Sex + Size + Gregarines + Sex: Gregarine + Sex: Size + Constant, Quasi (link = Log, variance =  $\mu$ );  
 Fecundity  $\sim$  Site + Size + Gregarines + Acariens + Size: Gregarines + Size: Acariens + Constant, Quasi (link = Log, variance =  $\mu$ );  
 Paired  $\sim$  Site + Size + Fecundity + Gregarines + Acariens + Size: Fecundity + Size: Gregarines + Size: Acariens + Fecundity: Gregarines + Fecundity:

Acariens + Constant, Quasi [link = logit, variance =  $\mu / (1 - \mu)$ ].

To test for possible assortative pairing between male and female *T. maritima* for body size or parasite load, and to remove confounding factors, we retrieved the residuals for paired male and female beetles from the relevant multiple regressions, which were:

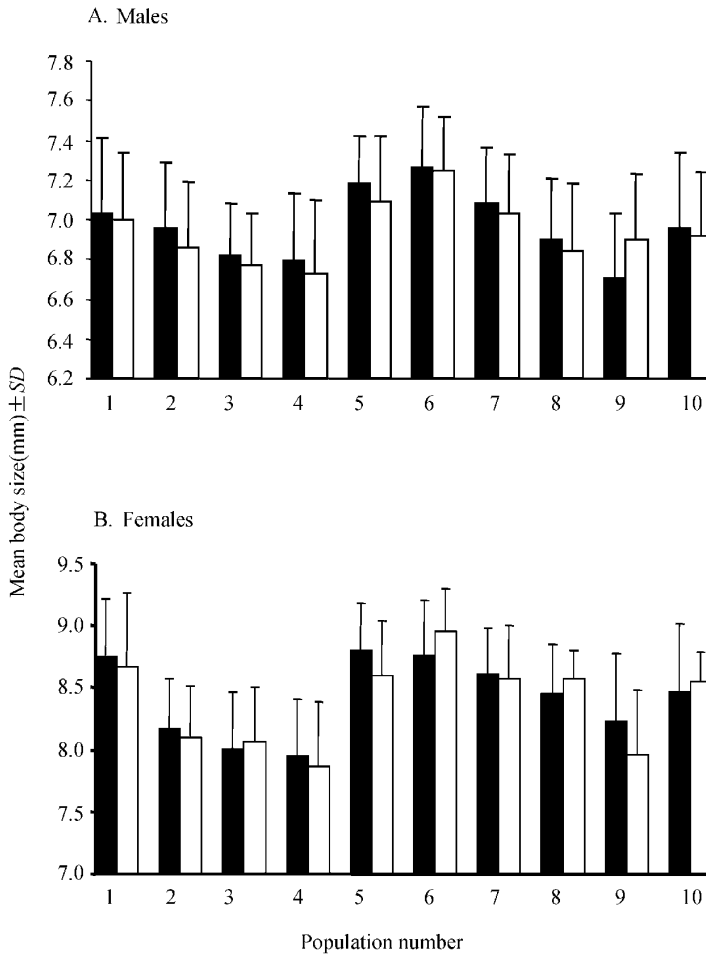
Size  $\sim$  Site + Constant, Gaussian;  
 Gregarines  $\sim$  Site + Constant, Quasi (link = Log, variance =  $\mu$ );  
 Acariens  $\sim$  Site + Size + Gregarines + Constant, Quasi (link = Log, variance =  $\mu$ );

For the correlation tests we used the Spearman correlation coefficient Rho.

For parasite abundances, the dispersion of data was measured with the aggregative coefficient  $\beta$ , which corresponds to the variance to mean ratio of parasite abundance across hosts (number of parasite individuals per host). This parameter is expected to be equal to 1 for a Poisson (random) distribution and above for an aggregated distribution. Mean crowding was computed according to the formula given in Reczigel (2005):

$$\bar{c} = \frac{\sum_{j=1}^n i_j^2}{\sum_{j=1}^n i_j}$$

where  $i_j$  is the number of parasites in host  $j$  and  $n$  is the total number of hosts examined.

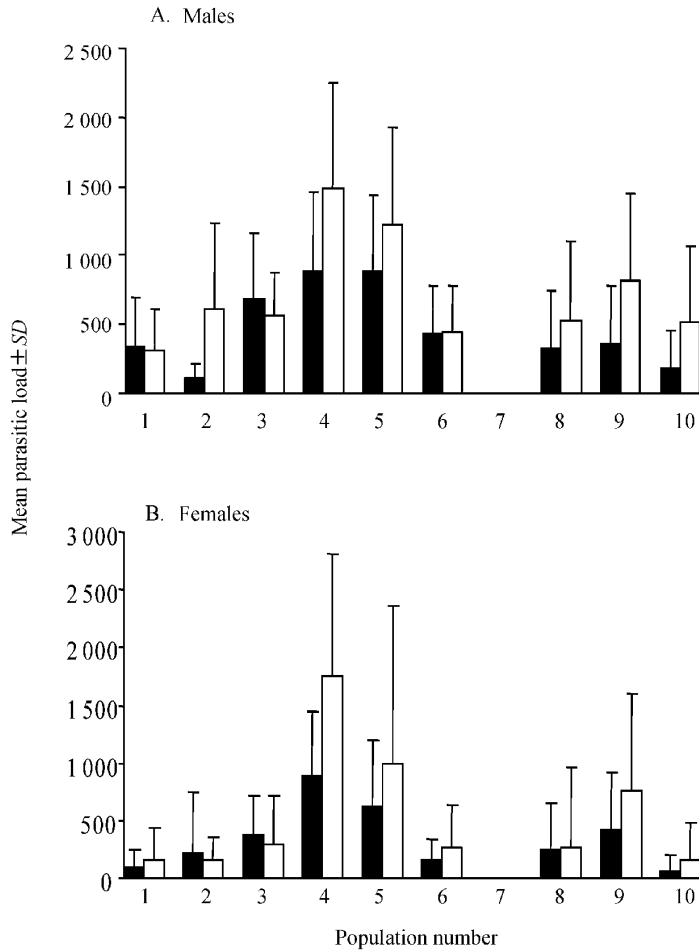


**Fig. 2 Mean body size  $\pm$  SD of males (A) and females (B) of *T. maritima* in the different populations sampled**  
 1: Quiberon; 2: Bretignole; 3: Gachere; 4: Aubraie; 5: Terriere; 6: Oleron; 7: Vieux Boucau; 8: Hossegor; 9: Capbreton; 10: Boucau. Dark bars: paired individuals, white bars: unpaired individuals.

## 2 Results

Variation in body size was largely (79%) explained by differences among sites and between genders of *T. maritima* individuals. The mean ( $\pm$  standard error) body size of females ( $8.42 \pm 0.02$ ) was much larger than that of males ( $6.95 \pm 0.01$ ) and this was heterogeneous across sites (Fig. 2). Both factors were highly significant ( $P < 0.0001$ ). Gregarine abundance was highly aggregated ( $\beta = 87.5$ ) and its variation was partially explained (38%) by site and beetle's sex. Males were more heavily infected by gregarines ( $513.6 \pm 18.91$ ) than females ( $377.5 \pm 22.19$ ), but most of the signal came from site differences (Fig. 3). In both cases, tests were highly significant ( $P < 0.0001$ ). The interaction between Sex and Size remained in the minimal model but was not significant ( $P = 0.08$ ), which is probably fortuitous because size did not explain anything when males and females were analyzed separately. Acarians were strongly aggregated ( $\beta = 9$ ). For gregarines the mean crowding was 1 239 and for acarians

it reached 15. The GLiM ( $R^2 = 0.67$ ) could not be simplified because of interactive terms that weakly explain the dispersion of data but were significant ( $P = 0.013$  for Sex: Size and  $P = 0.047$  for Sex: Gregarines). We therefore analyzed males and females separately. For females, 74% of the variance in mite load was explained almost exclusively by site ( $P < 0.0001$ ) and to a much lower extent (less than 1%) by *T. maritima* size with which acarain load increased ( $P = 0.002$ ). In male beetles, only Site was as important ( $P < 0.0001$ ) as gregarine load, which remained in the minimal model ( $R^2 = 0.53$ ), did not appear significant ( $P = 0.113$ ) and thus seemed marginally relevant if any. Correlation tests between residuals from Gregarine  $\sim$  Site + Sex + Size + Sex: Size and Acarians  $\sim$  Site + Sex + Size + Sex: Size showed no correlation between parasite and mite load ( $Rho = 0.04$ ,  $P = 0.12$ ). The most important factor explaining *T. maritima* fecundity was the site (partial  $R^2 = 0.43$ ,  $P < 0.0001$ , Fig. 4), but gregarine load had a negative effect on fecundity (partial  $R^2 =$



**Fig. 3** Mean parasitic load  $\pm$  SD of males (A) and females (B) of *T. maritima* in the different populations sampled 1: Quiberon; 2: Bretignole; 3: Gachere; 4: Aubraie; 5: Terriere; 6: Oleron; 7: Vieux Boucau; 8: Hossegor; 9: Capbreton; 10: Boucau. Dark bars: paired individuals, white bars: unpaired individuals.

0.04,  $P < 0.0001$ , Fig. 5) and beetle size a positive effect (partial  $R^2 = 0.02$ ,  $P < 0.0001$ ). The effect of gregarine abundance was more pronounced in the smallest *T. maritima*, which explains why the interaction term Size: Gregarines remained in the minimal model (partial  $R^2 < 0.01$ ,  $P = 0.0151$ ). Finally, pairing probability was poorly predictable from the minimal model ( $R^2 = 0.06$ ), but was heterogeneous across sites ( $P = 0.0034$ ), increased with fecundity ( $P = 0.0002$ ) and decreased with gregarine abundance ( $P = 0.0003$ ).

We found no correlation between female and male body size ( $Rho = 0.05$ ,  $P = 0.24$ ), a positive correlation between their gregarine loads (i.e., assortative pairing by parasitic load,  $Rho = 0.2$ ,  $P < 0.0001$ ) and no correlation between their acarian loads ( $Rho \sim 0$ ,  $P = 0.927$ ).

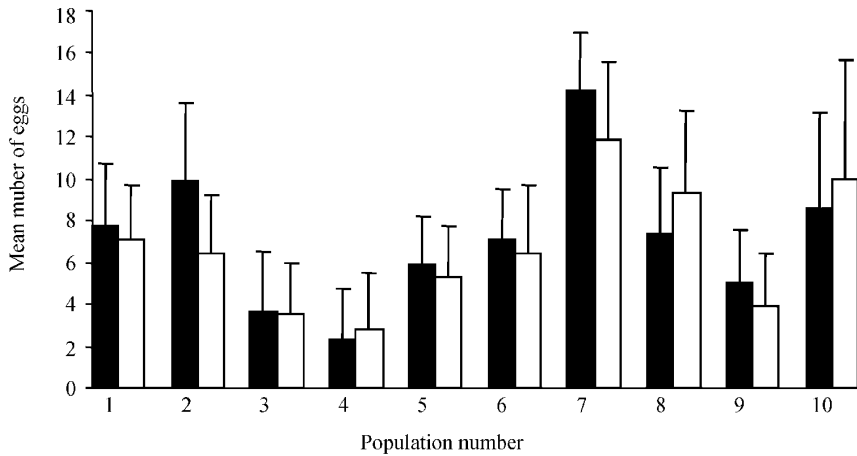
Sex ratio differed among populations (Fig. 6, Fisher exact test on Table r x k,  $P < 0.0001$ ). Only the insular population (6) has more females than males; elsewhere, males were always more abundant

than females but with wide variation (e.g., population 3 versus population 8).

### 3 Discussion

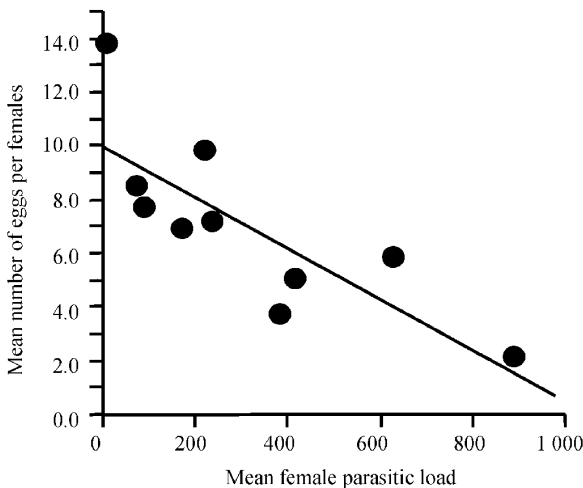
Despite occupying similar habitats at each of the 10 sites, significant differences of fecundity, adult body size, levels of parasitism, sex ratio and pairing pattern exist between populations of *T. maritima*. Whether these phenotypic variations are due to underlying genetic differences or from other phenomena (e.g., phenotypic plasticity) cannot be determined from this data set. Whatever the origin (s) of these differences, they are likely to interfere with the functioning of populations.

Parasitism by the acarian *P. chrysomelinus* has little effect on *T. maritima* fecundity. However, the protozoan parasite *G. munieri* is an important factor affecting the biology of *T. maritima*. The parasite is widespread (9 of 10 populations were infested) and has a major effect on the reproductive biology of its host. As in Thomas et al. (1999), we found that paired individuals were matched for parasitic load.



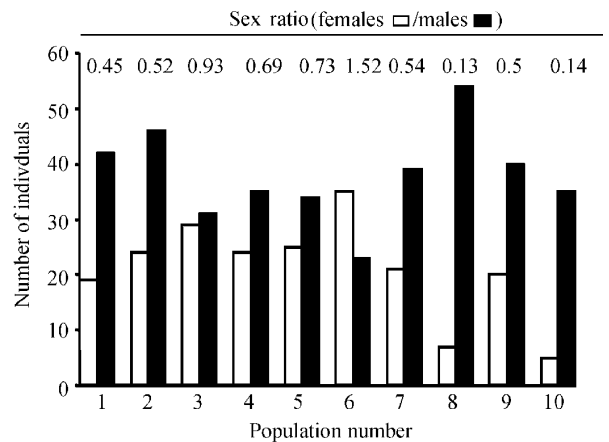
**Fig. 4** Mean number of eggs  $\pm$  SD produced by females of *T. maritima* in the different

1: Quiberon; 2: Bretignole; 3: Gachere; 4: Aubraie; 5: Terriere; 6: Oleron; 7: Vieux Boucau; 8: Hossegor; 9: Capbreton; 10: Boucau. Dark bars: paired individuals, white bars: unpaired individuals.



**Fig. 5** Relationship between the mean number of eggs produced and the mean parasitic load of females in the different populations sampled

This finding was the strongest in the most heavily infected population (4). These results, obtained 3 years after the first study, suggest that the original pair formation process that the parasite induces is stable in this beetle. Why males were more heavily infected than females is unclear but is in accordance with several studies suggesting a male-biased susceptibility to infection (e.g., Poulin 1996). Similarly, that heavily infected individuals are more likely to be unpaired than less infected ones is in accordance with the idea that parasites can affect mate choice processes and/or competitiveness (Hamilton and Zuk, 1982; Howard and Minchella, 1990). Finally, another major effect of *G. munieri* concerns its effect on female fecundity. Level of infection by gregarines can be considered as a significant determinant of the mean fecundity of females at the population level. Therefore, parasites are an important part of population ecology and should be included in studies wherever



**Fig. 6** Sex ratio in the different populations sampled

1: Quiberon; 2: Bretignole; 3: Gachere; 4: Aubraie; 5: Terriere; 6: Oleron; 7: Vieux Boucau; 8: Hossegor; 9: Capbreton; 10: Boucau. Dark bars: paired individuals, white bars: unpaired individuals.

possible.

Conversely to what is frequently observed in arthropods (see Crespi 1989 for review), there was no size assortative pairing in this beetle, even in parasite-free populations. In fact, it could be argued that given the weak link between size and fecundity in females, males preferring large females obtain no fitness benefits (at least in term of number of offspring). In such a context, we do not expect a preference for large females to evolve. Further studies would be necessary to understand which variable(s), in addition to parasites and female size, influence female fecundity.

The present work suggests that *T. maritima* displays a substantial phenotypic variability over its geographic range and that a significant part of this variability seems to be explained by the variation in infection level *G. munieri*. This work also suggests

that general conclusions about species ecology (including their interactions with parasites) must be considered with caution when derived from local and/or short term field studies. Consideration of the spatial structure as well as the heterogeneity of environmental conditions is highly desirable.

## References

- Bell MA, Foster SA, 1994. Introduction to the evolutionary biology of the threespine stickleback. In: Bell MA, Foster SA ed. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press, 1–27.
- Berthold P, 1993. *Bird Migration*. Oxford: Oxford University Press.
- Berthold P, 1999. Geographic variation and the microevolution of avian migratory behavior. In: Foster SA, Endler JA ed. *Geographic Variation in Behavior*. New York: Oxford University Press, 6–22.
- Borries C, Koenig A, Winkler P, 2001. Variation of life history traits and mating patterns in female langur monkeys *Semnopithecus entellus*. *Behav. Ecol. Sociobiol.* 50: 391–402.
- Chevin H, 1992. Contribution à la Biologie des *Timarcha* (col. Chrysomelidae). *L'entomologiste* 33: 133–141.
- Crespi BJ, 1989. Causes of assortative mating in arthropods. *Anim. Behav.* 38: 980–1 000.
- Endler JA, 1977. *Geographic Variation, Speciation and Clines*. Princeton, NJ: Princeton University Press.
- Endler JA, 1986. *Natural Selection in the Wild*. Princeton, NJ: Princeton University Press.
- Foster SA, Endler JA, 1999. *Geographic Variation in Behavior*. New York: Oxford University Press, 6–22.
- Guégan JF, Thomas F, Hochberg ME, de Meeus T, Renaud F, 2001. Disease diversity and human fertility. *Evolution* 55: 1 308–1 314.
- Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites. *Science* 218: 384–387.
- Hamilton WJ, Poulin R, 2001. Parasitism, water temperature and life history characteristics of the freshwater fish *Gobiomorphus breviceps* Stokell (Eleotridae). *Ecology of Freshwater Fish* 10: 105–110.
- Hanski I, 2002. *Metapopulation Ecology*. Oxford Series in Ecology and Evolution. Oxford: Oxford University Press.
- Harry OG, 1970. Gregarines: their effect on the growth of the desert locust *Schistocerca gregaria*. *Nature* 225: 964–966.
- Hewitt GM, Butlin RK, 1997. Causes and consequences of population structure. In: Krebs JR, Davies NB ed. *Behavioural Ecology: An Evolutionary Approach*. 4th edn. Oxford: Blackwell Science, Ltd., 350–372.
- Howard RD, Minchella DJ, 1990. Parasitism and mate competition. *Oikos* 58: 120–122.
- Johnson JB, 2001. Adaptive life-history evolution in the livebearing fish *Brachyrrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator-induced plasticity. *Evolution* 55: 1 486–1 491.
- Jolivet P, 1997. *Biologie des coléoptères chrysomelidés*. Boubée Paris.
- Lafferty KD, 1993. The marine snail *Cerithidea californica* matures at smaller sizes where parasitism is high. *Oikos* 68: 3–11.
- Poulin R, 1996. Sexual inequalities in helminth infections: a cost of being a male? *American Naturalist* 147: 287–295.
- Reiczigel J, Lang Z, Rozsa L, Tothmeresz B, 2005. Properties of crowding indices and statistical tools to analyse parasite crowding data. *Journal of Parasitology* 91: 245–252.
- Santiago-Blay JA, Fain A, 1994. Phoretic and ectoparasitic mites of the Chrysomelidae. In: Pierre J, Cox ML, Petitpierre E ed. *Novel Aspects of the Biology of Chrysomelidae*. Dordrecht, Boston, London: Kluwer Academic Publishers, 407–417.
- S-PLUS 2000 Guide to Statistics, Volume 1, Data Analysis Products Division, MathSoft, Seattle, WA.
- Thomas F, Oget E, Gente P, Desmots D, Renaud F, 1999. Assortative pairing with respect to parasite load in the beetle *Timarcha maritima* (Chrysomelidae). *J. Evol. Biol.* 12: 385–390.
- Thomas F, Renaud F, Benefice E, de Meeus T, Guégan JF, 2001. International variability of ages at menarche and menopause: patterns and main determinants. *Human Biology* 73: 271–290.
- Verrel PA, 1999. Geographic variation in sexual behavior. In: Foster SA, Endler JA ed. *Geographic Variation in Behavior*. New York: Oxford University Press, 6–22.
- Zuk M, 1987a. The effects of gregarine parasites on longevity weight loss, fecundity and developmental time in the field crickets *Gryllus veletis* and *G. pennsylvanicus*. *Ecol. Entomol.* 12: 349–354.
- Zuk M, 1987b. The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. *Behav. Ecol. Sociobiol.* 21: 65–72.