

Genetic Differentiation Among Populations of the Salt Marsh Beetle *Pogonus littoralis* (Coleoptera: Carabidae): A Comparison Between Atlantic and Mediterranean Populations

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Abstract

Genetic differentiation was studied among populations of the salt marsh beetle *Pogonus littoralis* (Coleoptera: Carabidae), comparing Atlantic and Mediterranean populations over a large part of its range. The genetic structure of this highly mobile beetle was investigated by studying allozyme polymorphism at nine enzyme loci in 13 populations. Mediterranean *P. littoralis* were highly significantly differentiated from Atlantic populations. Moreover, more isolated Atlantic populations showed increased differentiation and decreased genetic diversity compared to less fragmented Mediterranean populations.

The genetic structure of a species is influenced by its past history and by current gene flow. In Europe, the present-day patterns of phenotypic and genetic variation of many organisms have their origin in Pleistocene events (Hewitt 1999; Taberlet et al. 1998). After the retreat of the ice sheets since the last glacial maximum (about 18,000 years ago), northern Europe was subsequently reinvaded from several refuges in southern Europe. Under this scenario, populations in southern Europe should be genetically more diverse than populations that recently recolonized northern Europe, thus producing south-north clines of genetic variability (Eber and Brandl 1997). In theory, latitudinal differences in variability can be expected, as the colonizing populations are more prone to founder events and serial bottlenecks stemming from the greater demographic instability of these marginal populations. Moreover, not only past history, but also the current balance between gene flow, genetic drift, and selection shapes the observed genetic patterns of species. In small populations at the edge of a species range, genetic drift may lead to reduced within-population genetic diversity and increased between-population differentiation, while populations admixture

can lead to increased genetic diversity (Walter and Epperson 2001).

Another important factor in recent history is anthropogenic habitat fragmentation. Many areas of formerly continuous natural habitat have been subdivided into relatively small habitat islands surrounded by human-altered environments. Fragmentation generally results in reduced genetic diversity (Avice and Hamrick 1996; Frankham et al. 2002), but may also increase genetic differentiation between populations as a result of reduced gene flow (Slatkin 1994).

An excellent example of fragmented habitats are salt marshes. European salt marshes are relatively recent habitats, in many cases well documented historically. Present coastlines have only been in their current position for at most the last 6000 years (Adam 1990; Houthuys et al. 1993). Since their origin, the area of salt marshes in Europe has been considerably reduced due to geomorphological evolution and, in recent centuries, human activity (Schubel and Hirschberg 1978). Estimates all over western Europe show a recent and dramatic decrease in the surface area and habitat quality of salt marshes (Dijkema et al. 1984).

To investigate if an observed genetic differentiation results from fragmentation of the habitat or merely from a distance effect, the genetic population structure should be compared between continuous and more fragmented areas (e.g., Gibbs 1998; Knutsen et al. 2000; Peacock and Smith 1997; Van Dongen et al. 1998). These comparisons allow assessment of the impact of spatial habitat structure on population dynamics within species with specific life-history characteristics. However, these studies typically focus on species thought to have relatively low levels of vagility, possibly because fragmentation is more likely to disrupt gene flow in those species. Williams et al. (2003) were the first to identify genetic patterns consistent with recent habitat fragmentation in a wide-ranging, high gene flow butterfly on a large geographical scale.

Ground beetles (Coleoptera, Carabidae) appear to be ideal model organisms for such studies. The carabid beetle of this study, *Pogonus littoralis*, is a highly specialized halobiontic species, limited in its occurrence to salt marsh microhabitats, where it can reach relatively high densities (Desender 1989). This beetle has to be highly mobile because it is regularly forced to move between temporarily dry salt marsh ponds or creeks during its life cycle.

In this study we analyze the genetic diversity and differentiation of *P. littoralis* from the north to the south of its geographical distribution area using allozyme electrophoresis. The three main goals were (1) to investigate the distribution of genetic variation both within (genetic diversity) and among (genetic differentiation) Atlantic and Mediterranean populations; (2) to compare genetic diversity and differentiation among populations in continuous and more fragmented areas on a comparable geographical scale; and (3) to investigate genetic differentiation and variability in populations from two regions.

Materials and Methods

Studied Species and Sampling

Pogonus littoralis is a halobiont and highly mobile ground beetle, with a specialized microhabitat preference for unvegetated, temporarily dry salt marsh ponds or creeks, where it lives between cracks in humid sea clay. The geographical area of the species extends from the Mediterranean to western European Atlantic coast salt marshes, north to the British Isles and The Netherlands (Turin 2000). This constantly macropterous species shows maximally developed wings and functional flight musculature and is active during the day (Desender 1989).

Since 1993 we have collected *P. littoralis* beetles from five Atlantic (region with discontinuous or more fragmented distribution) and seven Mediterranean salt marshes (region with more continuous distribution) varying in size and isolation in France. We also sampled one population in Thessaloniki (Greece). All study sites are shown in Figure 1. In some areas, different populations could be studied. Beetles were collected by standardized hand-catches (unit of effort), mostly by using an aspirator, or by flotation whenever brackish water was available near a sampling site. Beetles were transported alive, and identified and counted with a binocular dissecting microscope. They were then frozen in liquid nitrogen or in an ultrafreezer until subjected to electrophoresis.

Allozyme Electrophoresis

Individuals were prepared for allozyme electrophoresis by homogenizing part of the body (head and thorax) in 40 μ l of distilled water on ice. Overall, more than 600 beetles, at least 40 per population (Table 1) were processed

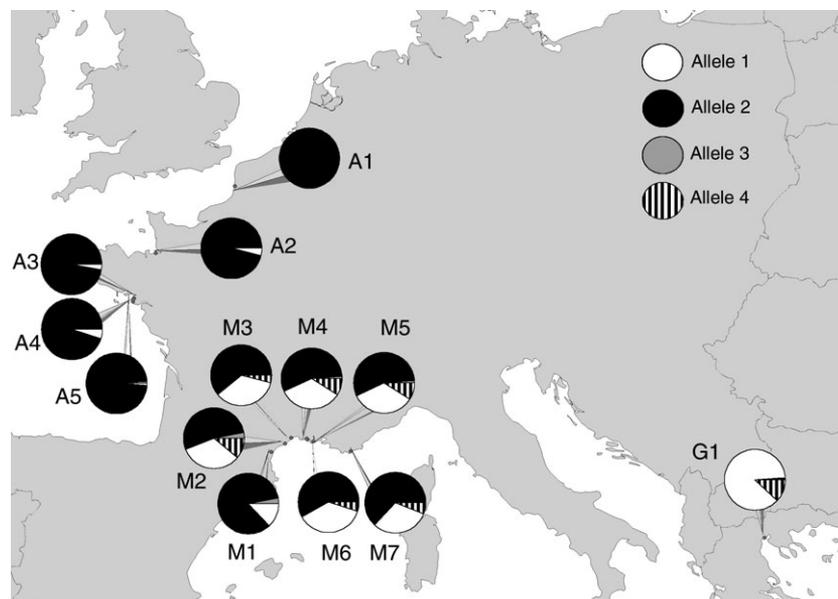


Figure 1. Study sites of *P. littoralis* and the geographical allele distribution of the polymorphic locus PEP-A1. For locality abbreviations, see Table 1.

Table 1. Within-population genetic diversity of 13 populations studied

Region	Sample number	Population name	<i>n</i>	<i>A</i>	<i>N</i>	<i>P</i>	<i>H_E</i>
France, Atlantic	A1	Authie	66	1.466 (0.291)	3	33.33%	0.067 (0.049)
	A2	Mont St Michel	43	2.281 (0.93)	6	66.67%	0.106 (0.055)
	A3	Guérande 1	39	2.111 (0.351)	6	66.67%	0.112 (0.054)
	A4	Guérande 2	40	1.889 (0.351)	5	55.56%	0.080 (0.051)
	A5	Guérande 3	39	1.889 (0.389)	4	44.44%	0.094 (0.055)
France, Mediterranean	M1	Bages	60	2.354 (0.318)	7	77.78%	0.215 (0.053)
	M2	Cap d'Agde	43	2.545 (0.437)	7	77.78%	0.273 (0.071)
	M3	Etang de Vic	38	2.778 (0.401)	8	88.89%	0.267 (0.068)
	M4	Camargue 1	87	2.506 (0.398)	7	77.78%	0.261 (0.069)
	M5	Camargue 2	40	2.556 (0.412)	8	88.89%	0.254 (0.067)
	M6	Camargue 3	40	2.333 (0.289)	8	88.89%	0.243 (0.062)
	M7	Toulon	45	2.432 (0.492)	7	77.78%	0.225 (0.073)
Greece	G1	Thessaloniki	49	1.585 (0.211)	4	44.44%	0.074 (0.045)

Standard errors are in parentheses. *n*, number of specimens; *A*, mean number of alleles over loci standardized between samples (*n* approximately 40, rarefaction); *N*, number of polymorphic loci; *P*, percentage of polymorphic loci; *H_E*, Nei's gene diversity. Sample numbers correspond to those in Figures 1 and 2.

for this study. Continuous cellulose acetate electrophoresis was carried out using standard methods (Hebert and Beaton 1989). Two buffer systems were used: Tris-glycine 10% (pH 8.5; Hebert and Beaton 1989) and Tris-maleate (pH 7.8; Richardson et al. 1986). Genotypes were scored for nine putative loci coding for seven enzymes: aldehyde oxidase (AO; EC 1.2.3.1), peptidase-A (dipeptide substrate: leucyl glycine, PEP-A1 and PEP-A2; EC 3.4.-.-), peptidase-Z (tripeptide substrate: leucyl glycine glycine, Pep-Z; EC 3.4.-.-), and phosphoglucomutase (PGM; EC 2.7.5.1) on a Tris-glycine buffer, and glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), malate dehydrogenase (MDH; EC 1.1.1.37), and isocitrate dehydrogenase 1 and 2 (IDH1, IDH2; EC 1.1.1.42) on a Tris-maleate buffer.

Data Analysis

Tests for Hardy-Weinberg equilibrium and linkage disequilibrium were performed with GENEPop (Raymond and Rousset 1995). Genetic diversity within populations was estimated using POPGENE (version 1.31; Yeh et al. 1997) in three ways: (1) the mean number of alleles over all loci, (2) the percentage of polymorphic loci, and (3) gene diversity: Nei's (1978) unbiased expected heterozygosity (*H_e*), averaged across loci. The mean number of alleles was standardized to samples of 40 individuals using the rarefaction technique (Hurlbert 1971). Differences between means were tested using STATISTICA (StatSoft Inc., Tulsa, OK). Genetic structure and differentiation were analyzed by evaluating among-population and among-region differentiation. Total genetic variance was therefore partitioned among regions, among populations within regions, and within populations by carrying out a hierarchical analysis of molecular variance (AMOVA) using ARLEQUIN (version 2.000; Schneider et al. 2000). GENETIX (Belkhir et al. 1996–1998) yielded *F_{ST}*

estimates (Weir and Cockerham 1984), tested for their significant departure from the null hypothesis (no differentiation) by means of a permutation procedure. Wright's *F_{ST}* was also calculated (Wright 1969). Genetic distances between populations were visualized in an unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on Nei's (1978) unbiased genetic distance.

Results

Genetic Variability

A total of 33 alleles were scored at nine loci across 13 populations. The number of alleles detected at each locus ranged from two (IDH1 and PEP-Z) to six (AO). Allele frequency tables for all loci are available upon request from the authors. No significant deviations from Hardy-Weinberg equilibrium were observed for any of the populations or loci. No significant linkage disequilibrium was found after applying the sequential Bonferroni correction. Therefore the studied loci can be interpreted as independent markers.

Within-Population and Within-Region Genetic Diversity

Genetic diversity varied a lot within regions, but there was a clear tendency for higher mean values in the Mediterranean area (Table 1): a Mann-Whitney *U*-test showed significantly higher values of mean expected heterozygosity (*U* = 0.0, *P* = .004) as well as mean alleles per locus (*U* = 0.0, *P* = .004) and mean percentage of polymorphic loci (*U* = 0.0, *P* = .004) for Mediterranean populations than for Atlantic ones. This higher variability is only to some degree caused by private alleles (three unique Atlantic alleles as compared to six for the Mediterranean; all in very low frequencies), but results especially from several non-unique alleles occurring at more equal frequencies and thus increasing heterozygosity in Mediterranean populations.

Inspection of the allele frequencies shows that a higher diversity in the Mediterranean samples is especially visible at the PEP-A1 locus (cf. Figure 1). The population from Greece has a much lower overall genetic diversity compared to the other Mediterranean samples from France.

Among-Population and Among-Region Genetic Differentiation

The overall F_{ST} value among all populations was 0.165 ($P < .0001$; Weir and Cockerham 1984). The Atlantic region F_{ST} was 0.023 ($P < .0030$) and the Mediterranean F_{ST} was 0.013 ($P < .0001$; sample of Greece not included). We also computed Wright's F_{ST} (Wright 1969) and obtained an overall F_{ST} of 0.161. Genetic differentiation estimates clearly increase at a larger geographic scale.

Based on Nei's (1978) genetic distances, we constructed a UPGMA dendrogram (Figure 2). All samples from Mediterranean France cluster together, as well as those from Atlantic France, whereas the sample from Thessaloniki forms a third group.

The major part of genetic variation was found within populations (75.36%), with 23.55% among regions and only 1.10% among populations within regions (AMOVA; Table 2). Exact tests showed a strong genetic differentiation among the three regions (all three comparisons $P < .001$). To test whether the Mediterranean France and Atlantic France groups really represent two differentiated regions, we excluded the Greek population from the AMOVA. Variance among regions dropped to 18.74%, but was still highly significant (Table 2). When the two regions (Mediterranean France and Atlantic France) were analyzed separately, most of the variation was again detected within populations, with up to 98.86% of the variance found within populations in Mediterranean France, but variance among populations was still highly significant (Table 2).

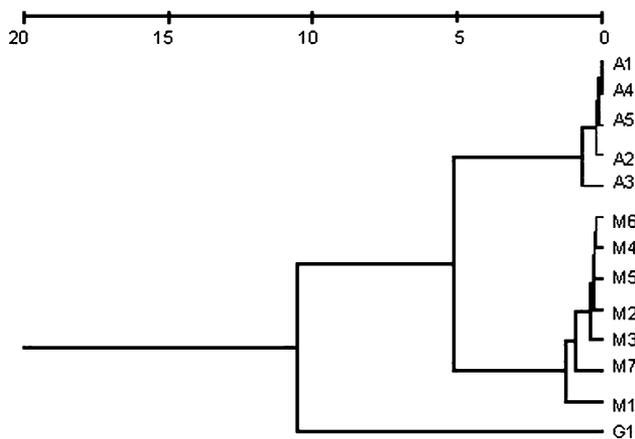


Figure 2. UPGMA dendrogram based on Nei's genetic distance for all studied populations in *P. littoralis*. For locality abbreviations, see Table 1.

Discussion

Allozyme Variability in Perspective

Significant genetic structuring (allozymes) has been reported for many insects, including beetles (Assmann and Weber 1997; De Jong et al. 2001; Desender et al. 1998; Desender and Serrano 1999; Desender and Verdyck 2001; Dhuyvetter et al. 2004; Epps et al. 2000; Hsiao 1989; Knoll and Rowell-Rahier 1998; Knoll et al. 1996; Knutzen et al. 2000). Estimates of overall genetic differentiation in the present study ($F_{ST} = 0.161$) are higher than the mean values (Wright's $F_{ST} = 0.103$) obtained for 30 other beetle species (Hsiao 1989), which are known to be among the highest recorded for insects (Ward et al. 1992). Next, F_{ST} values of *P. littoralis* are compared to those obtained in other salt marsh beetles. We only included comparable studies using both Atlantic and Mediterranean population samples to calculate an overall F_{ST} . Overall F_{ST} in the wing polymorphic *Pogonius chalcus* (see Desender and Serrano 1999; Dhuyvetter et al. 2004) appears to be larger, as expected, than in the related highly mobile *P. littoralis*. In *P. chalcus*, nearly 25% of the total observed genetic variation is due to differentiation among populations (Wright's F_{ST} ; Desender and Serrano 1999). Theories that relate variation in F_{ST} to variation in rates of gene flow indeed predict that species with a high dispersal power should show less population structuring (Waples 1998; Ward et al. 1992). In *Bembidion normannum*, another highly mobile salt marsh beetle, the overall F_{ST} is much lower than in *P. littoralis* (Wright's $F_{ST} = 0.059$; Desender and Verdyck 2001). The higher F_{ST} in *P. littoralis* is possibly due to the specific biology and microhabitat preference of this species (see further).

Population Differentiation

Mediterranean *P. littoralis* are highly significantly differentiated from Atlantic populations (Table 2 and Figure 2). Atlantic beetles of two other salt marsh species, *P. chalcus* and *B. normannum*, were also genetically distinct from Mediterranean populations (Desender and Serrano 1999; Desender and Verdyck 2001; Dhuyvetter et al. 2004). The highest gene diversity estimates of *P. littoralis* were found in the Mediterranean region and the lowest values were found in the Atlantic region (Table 1). This was also the case for *B. normannum* (Desender and Verdyck 2001), and is consistent with a much higher incidence of *P. littoralis* in Mediterranean salt marshes. The high genetic diversity observed within Mediterranean populations gives a strong indication that the evolutionary origin of *P. littoralis* lies in the Mediterranean area and/or that this area has served as glacial refugium, which was also suggested for *B. normannum* (Desender and Verdyck 2001).

We found that *P. littoralis* populations were genetically differentiated in both areas. However, the level of genetic differentiation among populations, as estimated by F_{ST} , was considerably greater in the Atlantic area, where the species shows a much more discontinuous distribution than in the Mediterranean area, where *P. littoralis* has a more

Table 2. Analysis of molecular variance based on nine allozyme loci in three regions: Atlantic France, Mediterranean France, and Greece^a

Source of variation	df	Sum of squares (SSD)	Variance components	Percent of the total variance	P value
Global analysis					
Among regions	2	166.028	0.23515	23.55	<0.001
Among populations within regions	10	17.961	0.01095	1.10	<0.001
Within populations	1245	936.924	0.75255	75.36	<0.001
Total	1257	1120.913	0.99865		
Atlantic France + Mediterranean France					
Among regions	1	103.615	0.18412	18.74	<0.001
Among populations within regions	10	17.961	0.01058	1.08	<0.001
Within populations	1148	904.434	0.78783	80.18	<0.001
Total	1159	1026.010	0.98254		
Atlantic France					
Among populations	4	4.541	0.00830	2.07	<0.001
Within populations	449	175.889	0.39173	97.93	<0.001
Total	453	180.430	0.400003		
Mediterranean France					
Among populations	6	13.420	0.01206	1.14	<0.001
Within populations	699	728.546	1.04227	98.86	<0.001
Total	705	741.966	1.05432		

^a Global analysis (three regions), analysis of populations from Atlantic France and Mediterranean France, and both regions analyzed separately.

continuous distribution. A similar result was obtained in another study comparing a fragmented area with a more continuous one in the highly mobile butterfly *Speyeria idalis* (Williams et al. 2003).

In the Mediterranean populations, we also observed a high level of genetic diversity within populations. This suggests that all populations are continuously exposed to gene flow. In view of the microhabitat preference of *P. littoralis*, populations in the Mediterranean area are probably to be viewed as metapopulations made up of ephemeral local populations. The resulting regular emigration (or local extinction) versus colonization dynamics is expected to enhance gene flow and consequently to continuously break down genetic differentiation, at least at a smaller geographic scale. In this area, the lowest within-population diversity was observed in the sample from Thessaloniki (Greece). This could be the result of genetic drift (random fixation of alleles) in less dense populations at the eastern edge of the species distribution. The population from Thessaloniki showed a three to eight times lower relative density compared to other Mediterranean samples. In metapopulation systems, edge populations have fewer neighbors than core populations. Fewer neighbors mean fewer immigrants, reduced chances of demographic rescue, and less gene flow (Segelbacher and Storch 2002). To understand this situation better in *P. littoralis*, we need to study some populations between France and Greece; for example, from Italy.

For the Atlantic populations, we suggest that *P. littoralis* has lost genetic variation due to founder events, population bottlenecks, or both. This interpretation is

supported by the finding that populations sampled in southern Europe are more variable than northern European populations, a finding consistent with the general tendency for populations in areas glaciated during the Pleistocene to exhibit reduced genetic variability (e.g., Merilä et al. 1996). Five of the nine studied enzymes are fixed or almost fixed in Atlantic populations compared to Mediterranean ones. Moreover, the observed elevated genetic differentiation is probably a direct consequence of genetic drift in conjunction with reduced or unbalanced dispersal following habitat fragmentation. Latitudinal differences in variability could be expected as the colonizing populations are prone to founder events and serial bottlenecks stemming from the greater demographic instability of these marginal populations. This is suggested by the allele frequencies of the PEP-A1 locus, where allele two is fixed in the northernmost population from the Authie estuary (Figure 1). We intend to study these patterns and suggested processes further in the near future, if possible by means of additional and more powerful genetic markers, such as recently developed microsatellite markers (Dhuyvetter and Desender 2003) or DNA sequencing.

Although the observed elevated genetic differentiation in the Atlantic region might in itself have limited consequences, the genome-wide implications are reduced population sizes and loss of possible advantageous alleles, as well as fixation of disadvantageous ones. This could therefore ultimately increase the chances of extinction, even for a species with high dispersal power. Distribution data for *P. littoralis* in several western European countries

indicate a significant regression of this salt marsh beetle during the past century (e.g., Desender et al. 1995; Hyman and Parsons 1992), as would be expected from the genetic data. It is, however, not yet clear whether habitat loss and fragmentation of Atlantic salt marshes or an overall decrease in their habitat quality is most responsible for this regression. In this context, we therefore plan to study some additional populations with more powerful markers.

Acknowledgments

This work was supported by the Entomology Department of the Royal Belgian Institute of Natural Sciences and the Federale Diensten voor Wetenschappelijke, Technische en Culturele Aangelegenheden (DWTC; project MO 36/006). It is carried out within the framework of the Flemish Research Network (FWO.010.97N, "Ecological genetics: patterns and processes of genetic variation in natural populations"; and FWO.017.02N, "Ecological genetics: a new approach"). A. Drumont assisted with part of the laboratory work. V. Versteirt and J.-Y. Rasplus helped in field sampling. T. Backeljau provided many helpful comments on the manuscript.

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Received April 22, 2004

Accepted November 6, 2004

Corresponding Editor: Ron C. Woodruff