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ORIGINAL PAPER

Genetic and morphological divergence at different spatiotemporal scales in the grasshopper *Mioscirtus wagneri* (Orthoptera: Acrididae)

Joaquín Ortego · Maria Pilar Aguirre · Pedro J. Cordero

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Abstract The study of the association between morphological and genetic divergence can provide important information on the factors determining population differentiation and gene flow at different spatiotemporal scales. In this study we analyze the congruence between morphological and genetic divergence in the Iberian populations of *Mioscirtus wagneri*, a specialized grasshopper exclusively inhabiting highly fragmented hypersaline low grounds. We have found strong morphological variation among the studied localities and among mtDNA- and microsatellite-based genetic clusters. However, we have detected some cases of morphological convergence between highly differentiated populations. By contrast, certain genetically homogeneous populations at both mtDNA and microsatellite markers showed significant morphological differentiation which may be explained by phenotypic plasticity or divergent selection pressures acting at different spatiotemporal scales. Mantel tests also revealed that morphological divergence was associated with microsatellite- but not with mtDNA-based genetic distances. Overall, this study suggests that morphological traits can provide additional information on the underlying population genetic structure when only data on scarcely variable mtDNA markers is available. Thus, morphology can retain useful information on genetic structure and has the benefit over molecular methods of being inexpensive, offering a preliminary/complementary useful criterion for the establishment of management units necessary to guide conservation policies.

Keywords Genetic differentiation · Microsatellites · mtDNA · Morphological divergence · Orthoptera

Introduction

Natural or human induced habitat fragmentation can reduce dispersal, increase genetic differentiation and erode the genetic diversity of remnant populations (Saunders et al. 1991; Frankham 1995; Frankham 1996; e.g. Vandergast et al. 2009). When populations become separated by effective barriers to gene flow increases the chance of phenotypic divergence (Smith et al. 1997; Garnier et al. 2005; Smith et al. 2005; Milá et al. 2009). Divergence can result from random genetic drift or as consequence of differential sexual (Panhuis et al. 2001) or natural selection (Schluter 2001) experienced by geographically separated populations (Barton 2001). Thus, restricted interbreeding often results in a phenotypic gradation of populations and this can ultimately reinforce reproductive isolation and speciation (Barton 2001; Turelli et al. 2001). For these reasons, the study of the factors determining phenotypic/genetic divergence is a central issue in evolutionary biology (Slatkin 1987).

The study of morphological divergence has important implications for basic and conservation research (Garnier et al. 2005; Nice and Shapiro 1999; Strange et al. 2008;

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J. Ortego (✉)
Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), C/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: joaquin.ortego@csic.es

M. P. Aguirre · P. J. Cordero
Grupo de Investigación de la Biodiversidad Genética y Cultural, Instituto de Investigación en Recursos Cinegéticos—IREC (CSIC, UCLM, JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain

Polihronakis 2009). If morphology reflects genetic differentiation, preliminary morphological surveys can motivate and guide future molecular based research on the factors (e.g. barriers to gene flow, differential selection, etc.) determining inter-population gene flow. If morphological differentiation parallels genetic divergence, phenotypic traits can also offer a valid criterion to establish management units when molecular data are not available (Garnier et al. 2005; García et al. 2008). Finally, morphological divergence can reveal cryptic patterns of incipient genetic differentiation which may have gone unresolved by nuclear markers (Nice and Shapiro 1999). This may occur if coding genes under strong directional selection are responsible for the observed phenotypic divergence and insufficient time has elapsed for reproductive isolation to be reflected as genetic divergence at neutral molecular markers (Greenberg et al. 1998; Nice and Shapiro 1999).

Mioscirtus wagneri (Orthoptera: Acrididae) is a highly specialized grasshopper exclusively inhabiting hypersaline low grounds with patches of *Suaeda vera*, the halophilic plant on which it exclusively depends for food and refuge (Cordero et al. 2007). *M. wagneri* shows a highly fragmented distribution and its Iberian populations have progressively become isolated due to historical and human-induced habitat reduction (Ortego et al. 2009; Ortego et al. 2010). Molecular-based research in the Iberian Peninsula has revealed strong genetic structure at both large and fine spatiotemporal scales. A mitochondrial DNA (mtDNA) based study has shown that the Iberian populations of *M. wagneri* present a marked phylogeographic structure, forming three main clades which correspond with populations located in northeast, central-southeast and southwest Iberia (Ortego et al. 2009). The higher resolution of microsatellite markers has also revealed a deep population substructure within these main mtDNA-based clades, with the presence of 7–11 genetic clusters which often involve close populations (Ortego et al. 2010). Such marked genetic structure is probably the result of the extremely fragmented distribution and isolation of their particular habitats (Ortego et al. 2009; Ortego et al. 2010) together with the scarce dispersal potential of this highly specialist species (J. Ortego, unpublished data). Thus, the marked genetic structure at both mtDNA and microsatellite markers makes this species an interesting model system to study the congruence between morphological and genetic differentiation at different spatiotemporal scales.

In this study we analyze the morphological variability of Iberian populations of *M. wagneri*, paying particular attention to the relationship between genetic and morphological divergence. For this purpose, we used previous molecular information to analyze morphological variability occurring at large (mtDNA; Ortego et al. 2009) and fine (microsatellites; Ortego et al. 2010) spatiotemporal scales. The particular objectives of the present study are: (1) to analyze the morphological differentiation within the distribution range of *M. wagneri* in the Iberian Peninsula; (2) to investigate the congruence between morphological and genetic structure inferred on the basis of both mtDNA and microsatellite markers; and (3) to test the null hypothesis that morphological divergence correlates with genetic divergence and geographic distance among populations. By answering these basic questions, we study whether simple and inexpensive morphological surveys can reflect genetic structure and be potentially useful to establish management units and guide conservation strategies.

Materials and methods

Sampling and study area

During 2006–2007, we sampled 24 populations of *M. wagneri*. We are confident these populations cover the entire species distribution range in the Iberian Peninsula, as several other potentially adequate habitats for *M. wagneri* (i.e. saline/hypersaline lagoons and low grounds) have been extensively prospected without any record of the species (Cordero et al. 2007; Ortego et al. 2009; Ortego et al. 2010). We collected 11–31 adult individuals per population and specimens were preserved whole in 1,500 µl of 96% ethanol at −20°C until needed for genetic and morphological analyses. We aimed to sample a similar number of males and females in each locality. However, male to female ratio is generally much higher than parity in this species (P. J. Cordero, unpublished data) and this generally resulted in smaller sample sizes for females in some sampling localities (Table 1). Population code description and further information on sampling locations are given in Table 1 and Fig. 1.

Morphology

For all individuals we measured femur and tibia length and maximum width of the hind leg, pronotum length, head length, and tegmen length to the nearest 0.1 mm using a stereoscopic microscope Leica S8 APO and the software LAS version 2.8.1. All these variables were strongly inter-correlated (all r values > 0.609; Table S1 in Supporting Information). For simplicity, all results presented in this article are only based on femur length, the body size estimate which was most strongly inter-correlated with all the other five measured variables (Table S1 in Supporting Information). We also estimated structural body size by performing a principal component analysis (PCA) on the six morphological traits. Following the broken-stick criterion (Jackson 1993), this PCA yielded one axis (PC1) which accounted for 96.33% of the total variance.

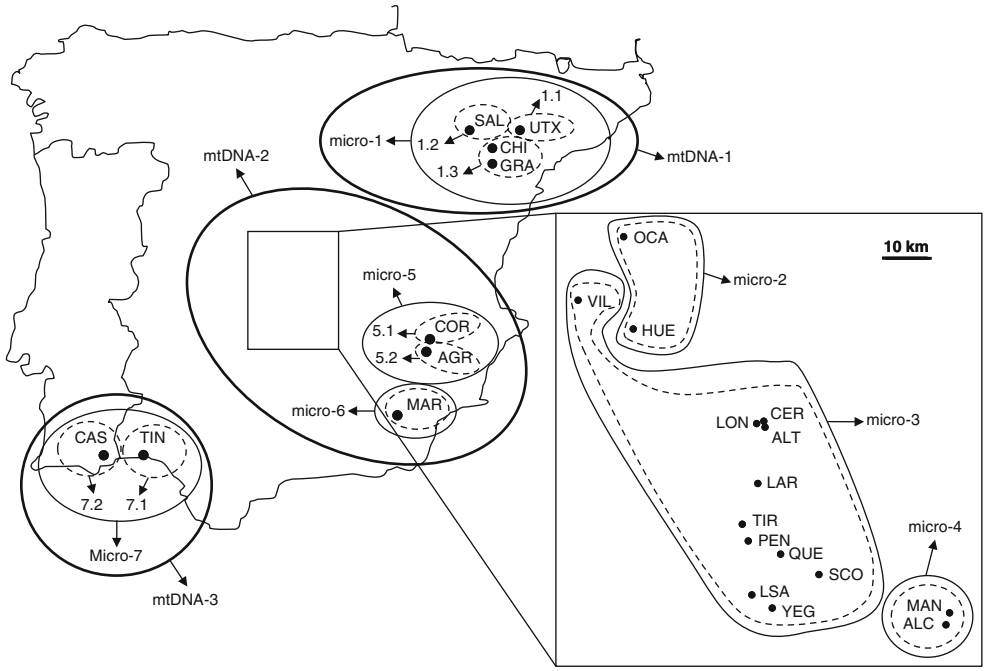

Fig. 1 Genetic clusters for the Iberian populations of *Mioscirtus wagneri* according with mitochondrial data (*thick solid lines*; Ortego et al. 2009) and microsatellite markers (*thin solid lines* for main clusters, *dashed lines* for secondary clusters; Ortego et al. 2010). Sampling sites and population codes are described in Table 1

This PCA yielded high factor loadings on the first principal component (>0.96 in all cases) and PC1 scores were used as an index of overall body size (e.g. Schauble 2004; Milá et al. 2009; Polihronakis 2009). We obtained analogous results using overall body size index (PC1) or femur length and the results for PC1-based analyses are only presented in Supporting Information (Tables S2–S3 and Fig. S1–S2).

Morphological, genetic, and geographical distances

Morphological divergence was calculated as the Euclidean distance between population mean values (e.g. Milá et al. 2009). Genetic divergence between sampling locations was calculated separately for microsatellite and mtDNA data using pairwise F_{ST} values and testing their significance with Fisher's exact tests after 10 000 permutations as implemented in Arlequin 3.1 (Excoffier et al. 2005). These datasets correspond to those used by Ortego et al. 2009 (for mtDNA; fragments of the genes 16S rRNA and cytochrome oxidase subunit II) and Ortego et al. 2010 (for microsatellite loci; Aguirre et al. 2010) to investigate the phylogeography and genetic structure of the Iberian populations of *M. wagneri* (Fig. 1). Geographical distances between populations were calculated as the straight-line distance between all pairs of sampling sites. We used Mantel tests to analyze the association between distance matrixes using zt software with 10 000 permutations (Bonnet and Van de Peer 2002).

Statistical analyses

We analyzed the association between body size and sex and population using General Linear Models (GLMs) in SPSS 17.0 software. Individuals were grouped on the basis of three criteria: (A) sampling locality, (B) mtDNA clusters, and (C) microsatellite clusters. In brief, we analyzed genetic structure for mtDNA and microsatellite data using Samova 1.0 (Dupanloup et al. 2002) and Structure 2.2 (Pritchard et al. 2000), respectively. In the analyses of microsatellite markers, we first pooled data from all populations to obtain main genetic clusters. Then, we re-analysed the data from the main clusters obtained separately to detect possible subtle genetic structure not revealed when all localities are pooled (e.g. Tzika et al. 2008; Ortego et al. 2010). Thus, we used both global and local analyses to cluster populations on the basis of microsatellite data (Fig. 1). More details for the analyses of genetic structure for both mtDNA and microsatellite data are indicated in Ortego et al. (2009) and (2010), respectively.

Results

Body size strongly differed among sampling localities and with mtDNA- and microsatellite-based clusters (Table 2; Fig. 2). We also found a strong interaction between sex and locality/cluster (Table 2). This interaction arises because

Table 1 Geographical location for the 24 studied populations of *Mioscirtus wagneri* in the Iberian Peninsula

Locality	Province	Code	Latitude	Longitude	*n* males	*n* females	mtDNA	Microsat
Northeast								
Pantano de Utxesa	Lleida	UTX	41°29′N	0°30′E	12	8	mtDNA-1	Micro-1.1
Laguna Salada	Zaragoza	SAL	41°30′N	0°43′W	17	3	mtDNA-1	Micro-1.2
Laguna de Chiprana	Zaragoza	CHI	41°14′N	0°11′W	12	6	mtDNA-1	Micro-1.3
Laguna Salada Grande	Teruel	GRA	41°02′N	0°12′W	18	2	mtDNA-1	Micro-1.3
Central-Southeast								
Saladar de Ocaña	Toledo	OCA	39°58′N	3°38′W	9	11	mtDNA-2	Micro-2.1
Saladar de Huerta	Toledo	HUE	39°50′N	3°37′W	8	10	mtDNA-2	Micro-2.1
Saladar de Villasequilla	Toledo	VIL	39°53′N	3°44′W	7	11	mtDNA-2	Micro-3.1
Laguna del Cerrillo	Toledo	CER	39°41′N	3°18′W	10	10	mtDNA-2	Micro-3.1
Laguna del Altillo	Toledo	ALT	39°41′N	3°18′W	9	11	mtDNA-2	Micro-3.1
Laguna de Longar	Toledo	LON	39°41′N	3°19′W	10	10	mtDNA-2	Micro-3.1
Laguna Larga	Toledo	LAR	39°36′N	3°18′W	16	4	mtDNA-2	Micro-3.1
Laguna de Tírez	Toledo	TIR	39°32′N	3°21′W	10	10	mtDNA-2	Micro-3.1
Laguna de Peña Hueca	Toledo	PEN	39°31′N	3°20′W	9	11	mtDNA-2	Micro-3.1
Laguna de Quero	Toledo	QUE	39°29′N	3°15′W	18	5	mtDNA-2	Micro-3.1
Laguna de la Sal	Toledo	LSA	39°26′N	3°20′W	9	11	mtDNA-2	Micro-3.1
Laguna de las Yeguas	Toledo	YEG	39°25′N	3°17′W	10	10	mtDNA-2	Micro-3.1
Laguna de Salicor	Ciudad Real	SCO	39°27′N	3°10′W	21	10	mtDNA-2	Micro-3.1
Laguna de Alcahozo	Ciudad Real	ALC	39°23′N	2°52′W	10	10	mtDNA-2	Micro-4.1
Laguna de Manjavacas	Cuenca	MAN	39°24′N	2°52′W	11	7	mtDNA-2	Micro-4.1
Saladar de Cordovilla	Albacete	COR	38°33′N	1°38′W	17	3	mtDNA-2	Micro-5.1
Saladar de Agramón	Albacete	AGR	38°24′N	1°37′W	9	2	mtDNA-2	Micro-5.2
Saladar del Margen	Granada	MAR	37°38′N	2°34′W	9	11	mtDNA-2	Micro-6.1
Southwest								
Río Tinto	Huelva	TIN	37°13′N	6°54′W	11	6	mtDNA-3	Micro-7.1
Castro Marin	Algarve	CAS	37°14′N	7°30′W	13	7	mtDNA-3	Micro-7.2

Genetic clusters for mtDNA and microsatellite markers are indicated

body size divergence differed between sexes in several pair-wise population comparisons (Fig. 2). Although these analyses suggest a main genetic-morphological congruence, detailed pair-wise population comparisons revealed that several genetically differentiated populations showed no significant body size differentiation (Table 3; Fig. 2). By contrast, some genetically homogeneous populations showed significant morphological divergence (Table 3; Fig. 2). Morphology was more geographically structured than mtDNA variability, i.e. genetically homogeneous populations at mtDNA markers generally showed strong morphological differentiation (Fig. 2; Table 3). We also found that morphology was more geographically structured than microsatellite variability in some localities, particularly those involving the extensively sampled populations from Central Spain (Fig. 2; Table 3). Morphological divergence was strongly correlated with geographical distance (Mantel tests, males: $r = 0.215$, $P < 0.001$; females:

Table 2 GLMs for the association between body size (femur length) and sex and locality/genetic cluster

Model	Test	*P*
(A) Sampling localities		
Sex	$F_{1,\ 426} = 5801.66$	<0.001
Locality	$F_{23,\ 426} = 17.78$	<0.001
Sex × Locality	$F_{23,\ 426} = 2.98$	<0.001
(B) mtDNA clusters		
Sex	$F_{1,\ 468} = 1617.99$	<0.001
mtDNA cluster	$F_{2,\ 468} = 28.33$	<0.001
Sex × mtDNA cluster	$F_{2,\ 468} = 5.66$	0.004
(C) Microsatellite clusters		
Sex	$F_{1,\ 460} = 2507.13$	<0.001
Microsatellite cluster	$F_{6,\ 460} = 31.53$	<0.001
Sex × Microsatellite cluster	$F_{6,\ 460} = 4.10$	0.001

Individuals were grouped on the basis of three criteria: (A) sampling locality, (B) mtDNA clusters, and (C) microsatellite clusters

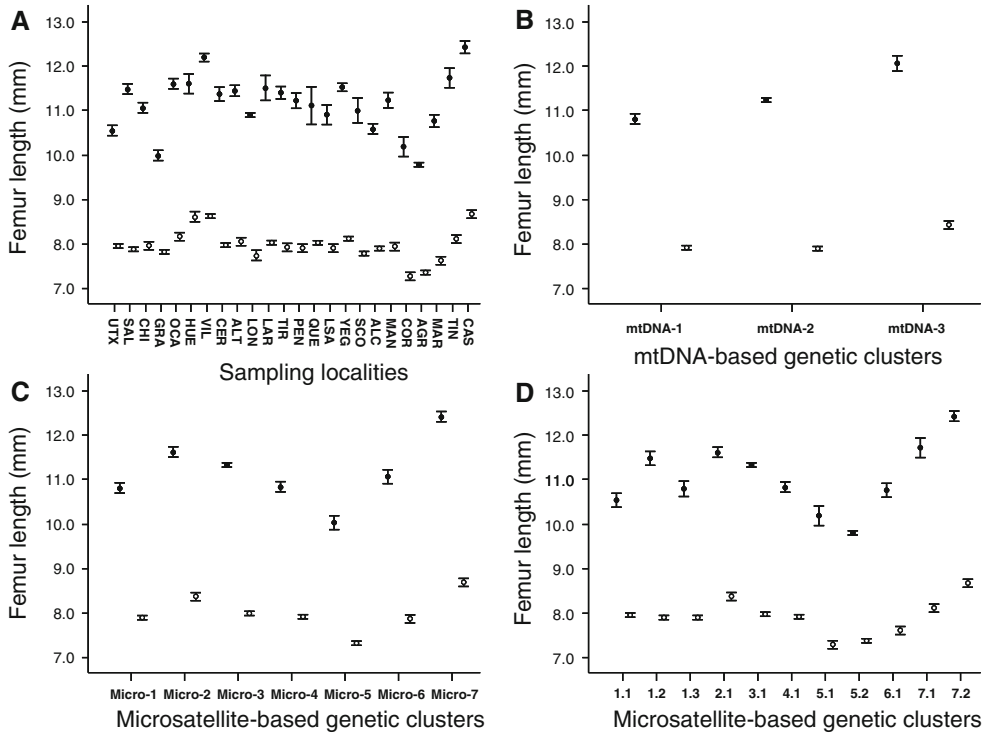

Fig. 2 Body size (femur length) in relation with sex (males: *open circles*; females: *solid circles*) and populations. Populations were grouped on the basis of three criteria: **a** sampling locality, **b** mtDNA-based clusters, and **c**, **d** microsatellite-based clusters. Population and cluster codes are described in Table 1

$r = 0.364$, $P < 0.001$). We found no association between morphological and mtDNA divergence (Mantel tests, males: $r = -0.085$, $P = 0.134$; females: $r = 0.101$, $P = 0.066$; Fig. 3a). However, there was a strong association between morphological and microsatellite divergence (Mantel tests, males: $r = 0.165$, $P = 0.010$; females: $r = 0.250$, $P < 0.001$; Fig. 3b).

Discussion

We found significant morphological differentiation among Iberian populations of the grasshopper *M. wagneri*, suggesting that selection and/or genetic drift together with long-term population isolation has probably contributed to morphological divergence at different spatiotemporal scales (Garnier et al. 2005; García et al. 2008; Polihronakis 2009). Accordingly, we have found a strong morphological divergence between most genetic clusters obtained from previous mtDNA and microsatellite analyses on spatial genetic structure. However, morphological divergence correlated with genetic differentiation at microsatellite markers but such association was not statistically significant for mtDNA. The latter has probably resulted from the lower resolution of mtDNA in comparison with microsatellites markers, i.e. only three mtDNA genetic clusters have been found in the Iberian Peninsula (Ortego et al. 2009) whereas microsatellite analyses revealed the presence of 7–11 genetically differentiated groups (Ortego et al. 2010). Thus, the marked morphological divergence observed at moderately fine spatiotemporal scales seems to be not well reflected by the scarcely variable mtDNA markers used to characterize the studied populations (Milá et al. 2009; Polihronakis 2009).

Despite the general correspondence between body size and genetic divergence, morphology was not completely congruent with genetic data (e.g. Greenberg et al. 1998; Lee and Frost 2002; Illera et al. 2007). Some genetically differentiated populations showed no morphological divergence, suggesting stabilizing selection or convergent evolutionary pressures in certain distant populations (Lee and Frost 2002). By contrast, we have found absence of morphological divergence between some geographically close populations with low or disrupted gene flow (e.g. SAL-CHI; Fig. 1). Such absence of morphological divergence could have resulted from morphological stasis for the studied traits due to stabilizing/fluctuating selection in geographically close populations experiencing similar variable environmental conditions (Charlesworth et al. 1982; e.g. Lee and Frost 2002; Toju and Sota 2009). Finally, some genetically homogeneous populations showed significant morphological divergence (e.g. some

Table 3 Pair-wise population values of morphological divergence (femur length) for males (above the diagonal) and females (below the diagonal)

mtDNA	**1**				**2**																			**3**	
Micro	**1**				**2**		**3**												**4**		**5**		**6**	**7**	
Micro	**1.1**	**1.2**	**1.3**		**2**		**3**												**4**		**5.1**	**5.2**	**6**	**7.1**	**7.2**
	UTX	SAL	CHI	GRA	OCA	HUE	VIL	CER	ALT	LON	LAR	TIR	PEN	QUE	LSA	YEG	SCO	ALC	MAN	COR	AGR	MAR	TIN	CAS	
UTX	–	0.227	0.045	0.069	0.068	**0.548**	**0.625**	0.054	0.023	0.184	0.076	0.073	0.114	0.026	0.111	0.105	0.314	0.111	0.086	**0.794**	**0.569**	0.432	0.093	**0.664**	
SAL	–	–	0.272	0.296	0.295	**0.775**	**0.852**	0.174	0.250	0.043	0.303	0.154	0.113	0.201	0.116	0.332	0.087	0.116	0.141	**0.566**	**0.342**	0.205	0.320	**0.891**	
CHI	0.347	–	–	0.025	0.023	**0.503**	**0.580**	0.098	0.022	0.229	0.031	0.117	0.159	0.071	0.156	0.060	0.359	0.155	0.130	**0.838**	**0.614**	0.476	0.049	**0.619**	
GRA	–	–	–	–	0.001	**0.479**	**0.556**	0.123	0.046	0.253	0.007	0.142	0.183	0.095	0.180	0.035	0.383	0.180	0.155	**0.863**	**0.638**	0.501	0.024	**0.595**	
OCA	**0.960**	–	0.614	–	–	0.480	0.557	0.122	0.045	0.252	0.008	0.141	0.182	0.094	0.179	0.037	0.382	0.179	0.154	**0.861**	**0.637**	**0.500**	0.025	**0.596**	
HUE	**0.465**	–	0.118	–	0.495	–	0.077	**0.601**	**0.525**	**0.732**	**0.472**	**0.620**	**0.662**	**0.574**	**0.659**	0.443	**0.862**	**0.658**	**0.633**	**1.341**	**1.117**	**0.979**	**0.454**	0.116	
VIL	**1.426**	–	**1.080**	–	0.466	0.961	–	**0.678**	**0.602**	**0.809**	**0.549**	**0.697**	**0.739**	**0.651**	**0.736**	**0.520**	**0.939**	**0.735**	**0.710**	**1.418**	**1.194**	**1.056**	**0.531**	0.039	
CER	0.666	–	0.319	–	0.294	0.201	**0.761**	–	0.077	0.131	0.129	0.019	0.060	0.027	0.058	0.158	0.260	0.057	0.032	**0.740**	**0.516**	0.378	0.147	**0.717**	
ALT	**0.732**	–	0.385	–	0.229	0.267	0.695	0.066	–	0.207	0.053	0.096	0.137	0.049	0.134	0.082	0.337	0.134	0.109	**0.816**	**0.592**	0.455	0.070	**0.641**	
LON	0.162	–	0.185	–	0.798	0.303	**1.265**	0.504	0.570	–	0.260	0.112	0.070	0.158	0.073	0.289	0.130	0.074	0.099	**0.609**	0.385	0.247	0.278	0.848	
LAR	–	–	–	–	–	–	–	–	–	–	–	0.149	0.190	0.102	0.187	0.029	0.390	0.187	0.162	**0.869**	**0.645**	0.508	0.017	**0.588**	
TIR	0.661	–	0.314	–	0.299	0.196	0.766	0.005	0.071	0.499	–	–	0.041	0.046	0.039	0.177	0.241	0.038	0.013	**0.721**	**0.496**	0.359	0.166	**0.736**	
PEN	0.472	–	0.126	–	0.488	0.007	**0.954**	0.193	0.259	0.311	–	0.188	–	0.088	0.003	0.219	0.200	0.003	0.028	**0.679**	**0.455**	0.318	0.207	**0.778**	
QUE	0.353	–	0.007	–	0.607	0.112	**1.073**	0.312	0.378	0.191	–	0.307	0.119	–	0.085	0.131	0.288	0.084	0.059	**0.767**	**0.543**	0.405	0.120	**0.690**	
LSA	0.048	–	0.395	–	1.008	0.513	**1.475**	0.714	0.780	0.210	–	0.709	0.521	0.402	–	0.216	0.203	0.001	0.026	**0.682**	**0.458**	0.320	0.205	**0.775**	
YEG	**0.778**	–	0.432	–	0.182	0.313	0.648	0.113	0.047	0.616	–	0.118	0.306	0.425	0.826	–	0.419	0.215	0.190	**0.898**	**0.674**	**0.536**	0.011	**0.559**	
SCO	0.528	–	0.182	–	0.432	0.063	**0.898**	0.137	0.203	0.366	–	0.132	0.056	0.175	0.577	0.250	–	0.203	0.228	**0.480**	0.255	0.118	0.407	**0.978**	
ALC	0.188	–	0.535	–	**1.149**	**0.653**	**1.615**	0.854	**0.920**	0.350	–	0.849	0.661	0.542	0.140	0.967	0.717	–	0.025	**0.683**	**0.459**	0.321	0.204	**0.774**	
MAN	0.488	–	0.141	–	0.473	0.023	**0.939**	0.178	0.244	0.326	–	0.173	0.015	0.134	0.536	0.291	0.041	0.676	–	**0.708**	**0.483**	0.346	0.179	**0.749**	
COR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.224	0.362	**0.887**	**1.457**	
AGR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.137	**0.662**	**1.233**	
MAR	0.189	–	0.536	–	**1.149**	**0.654**	**1.616**	0.855	0.921	0.351	–	0.850	0.662	0.543	0.141	0.968	0.718	0.001	0.677	–	–	–	**0.525**	**1.095**	
TIN	**0.969**	–	0.622	–	0.009	0.504	0.457	0.303	0.237	0.807	–	0.308	0.497	0.616	1.017	0.191	0.441	**1.158**	0.481	–	–	1.158	–	**0.570**	
CAS	**1.666**	–	**1.320**	–	0.706	1.201	0.240	**1.001**	0.935	**1.504**	–	**1.006**	**1.194**	**1.313**	**1.715**	0.888	**1.138**	**1.855**	**1.179**	–	–	**1.856**	0.697	–	

Values in bold are statistically significant after sequential Bonferroni corrections ($P < 0.05$)

Only localities with five or more sampled individuals for each sex are indicated

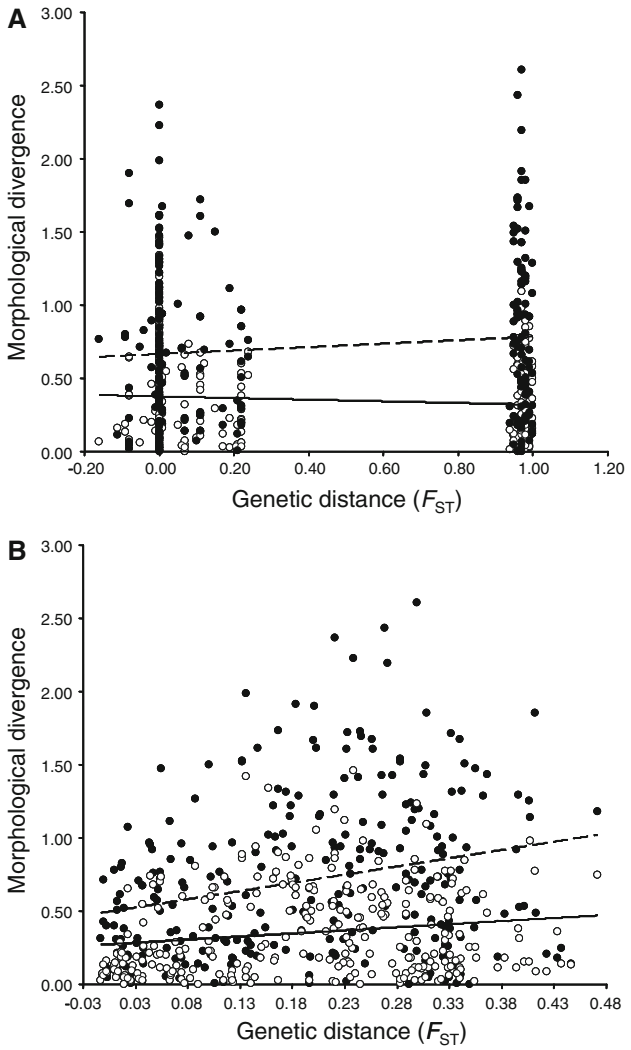

Fig. 3 Correlation between genetic (F_{ST}) and morphological divergence (femur length) for the Iberian populations of *Mioscirtus wagneri* considering **a** mtDNA and **b** microsatellite markers. Males: *open circles*, *solid lines*; Females: *filled circles*, *dashed lines*

populations from La Mancha region, Central Spain; Fig. 1). In this case, morphological differentiation could have resulted from phenotypic plasticity or ongoing divergent evolutionary pressures operating at very fine spatial scales (Greenberg et al. 1998; Nice and Shapiro 1999). At such spatiotemporal scale, the disruption of gene flow may be so recent that not even highly variable microsatellite markers are reflecting it (Greenberg et al. 1998; Nice and Shapiro 1999). As it has been suggested in other systems, this could have important evolutionary implications particularly if morphological divergence at such fine spatiotemporal scales generates reproductive isolation and this further contributes to reduce inter-population gene flow (Funk et al. 2009; Wang and Summers 2010). Natural/sexual selection may also underlay the observed sexual differences in body size divergence: sex-specific morphological differentiation could be revealing divergent evolutionary pressures in both sexes which are not reflected by genetic differentiation at autosomal markers or scarcely variable maternally inherited mtDNA genes. Thus, although body size in Orthoptera shows moderate to high heritability, this trait is under strong natural selection and this may explain the discordance between neutral genetic differentiation and morphological divergence observed in some populations (see Whitman 2008 for a review).

Overall, this study provides evidence that both mtDNA and microsatellite markers reflect evolutionary changes occurring at different spatiotemporal scales. Detailed analyses considering other traits could also help to reveal cryptic patterns of morphological divergence and reproductive isolation uncovered in this study (e.g. genitalia morphology; Garnier et al. 2005; Polihronakis 2009). Future studies analyzing the environmental factors which affect morphological divergence (Telfer and Hassall 1999; Heidinger et al. 2010) together with common garden experiments (Telfer and Hassall 1999) are necessary to determine the relative role of selection and drift on the patterns of morphological divergence observed. This would also help to resolve which percentage of the morphological variance is merely due to phenotypic plasticity (Pigliucci 2001; Lee and Frost 2002; Ramírez-Valiente et al. 2009). Although some apparently significant morphological variation could be misleading, our study suggests that additional morphological information can help to resolve evolutionary divergence which is not well reflected by scarcely variable mtDNA markers, Thus, morphology can retain useful information on genetic structure and has the benefit over molecular methods of being inexpensive, offering a preliminary/complementary useful criterion for the establishment of management units necessary to guide conservation policies.

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