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SHORT COMMUNICATION

Tracking the origin of an invasive species: *Drosophila subobscura* in Argentina

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Abstract

Biological invasions are excellent opportunities to study the evolutionary forces leading to the adaptation of a species to a new habitat. Knowledge of the introduction history of colonizing species helps tracking colonizing routes and assists in defining management strategies for invasive species. The Palearctic species *Drosophila subobscura* is a good model organism for tracking colonizations since it was detected in Chile and western North America three decades ago and later on in the Atlantic coast of Argentina. To unravel the origin of the Argentinean colonizers two populations have been analysed with several genetic markers. Chromosomal arrangements and microsatellite alleles found in Argentina are almost similar to those observed in Chile and USA. The lethal allelism test demonstrates that the lethal gene associated with the O_5 inversions in Argentina is identical to that found in Chile and USA, strongly supporting the hypothesis that all the American colonizing populations originated from the same colonization event. A secondary bottleneck is detected in the Argentinean populations and the genetic markers suggest that these populations originated from the invasion of 80–150 founding individuals from Chile.

Introduction

The invasion of a geographical area by a new species can be regarded as an evolutionary natural experiment (Sax *et al.*, 2007). Biological invasions are considered magnificent models for rapid evolution (Lee, 2002). In the last 50 years global trade increase and climate change have drastically favoured invasions of both marine and terrestrial taxa (Lee, 2002; Rius *et al.*, 2008). Unveiling the introduction history of colonizing species helps tracking colonizing routes and assists in defining management strategies for invasive species. Furthermore, knowing the origin of colonizers can favour studying the properties that allow a species to succeed in a novel habitat and the constraints that limit range expansion (Gilchrist & Lee, 2007). The success of colonizing species may depend on their ability to evolve in response to their new environment, promoting its success in highly disturbed, human-dominated landscapes. However, colonizing species may evolve, both during their initial establishment and during subsequent range expansion, in response to selection pressures (e.g. Lee, 2002; Balanyà *et al.*, 2006). Multiple introductions can often be critical to the successful establishment and spread of introduced species, as they may be important sources of genetic variation necessary for adaptation in new environments. To understand the evolutionary genetics of colonizations, one must identify the most likely source of colonizers, the levels of genetic diversity of both introduced and native populations, the geographical pathways of spread and the ability of the populations to evolve in novel environments.

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Drosophila subobscura Collin is an excellent model organism for addressing these questions. It was considered to be a typical Palearctic species; however, in 1978, it was detected for the first time in southern South America (Puerto Montt, Chile) (Brncic & Budnik, 1980), and, in 1982, in western North America (Port Townsend, Washington, USA) (Beckenbach & Prevosti, 1986). Colonization was rapid and successful both in Chile and in North America (USA and Canada), and presently the species is detected in an area ranging from 29° to 53°S in Chile and from 35° to 55°N in western North America (Brncic *et al.*, 1981; Ayala *et al.*, 1989). The success of this colonization is likely related to the striking climatic similarity of both regions to the original Palearctic species region with gradual variations from marine European Atlantic west coast to Mediterranean and steppe-desert climate (Ayala *et al.*, 1989; Prevosti *et al.*, 1989). Likewise, this climatic similarity is also reflected by a convergence in natural vegetation and cultivated plants (Prevosti *et al.*, 1989). Nevertheless there is a significant difference in *Drosophila* fauna between North and South America. While the *Drosophila* fauna in South America includes no species of the *obscura* group, to which *D. subobscura* belongs (Brncic *et al.*, 1985), in western North America there are a number of obscura group species, including *D. pseudoobscura*, *D. persimilis*, *D. miranda*, *D. athabasca* and *D. azteca* (Ayala *et al.*, 1989; Prevosti *et al.*, 1989). Interestingly these species could be potential competitors of *D. subobscura* colonizers (Pascual *et al.*, 1998, 2000a).

Several lines of evidence revealed that the colonization of Chile and USA resulted from a single colonizing stock (Prevosti *et al.*, 1989; Mestres *et al.*, 1990, 2005; Pascual *et al.*, 2007). First, *D. subobscura* colonizing populations share the same chromosomal arrangements (Prevosti *et al.*, 1988) with the exception of some putative new inversions that were eliminated soon after colonization (Balanyà *et al.*, 2003). Secondly, a complete association between a lethal gene and the O_5 inversion was found in all Chilean and North American populations analysed so far (Mestres *et al.*, 1992, 1995; Solé *et al.*, 2000). This association has probably persisted due to the heterotic effect of the New World O_5 inversion (Mestres *et al.*, 2001). Furthermore, other lethal genes (i.e. those presenting an incomplete association with O_{3+4+2} and O_{3+4+7}) are shared in both hemispheres, confirming that both colonizations are strongly related (Mestres *et al.*, 1992, 1995, 2005, 2008; Solé *et al.*, 2000). Finally, Approximate Bayesian Computation methods on microsatellite data from South American, North American and European populations give the strongest support to the nonindependence of both colonizing events and to a scenario of successive founder events first from Europe into South America, and later from South America into North America (Pascual *et al.*, 2007).

The number of founders that reached America from Europe was estimated to be between 4 and 150 (Brncic *et al.*, 1981; Mestres *et al.*, 1990; Pascual *et al.*, 2001, 2007). The founder effect is reflected by a loss of genetic variability in colonizing populations: they present a reduced number of chromosomal arrangements (Prevosti *et al.*, 1985), a lower number of alleles in allozyme loci (Balanyà *et al.*, 1994), a reduced number of haplotypes in both mtDNA (Latorre *et al.*, 1986; Rozas *et al.*, 1990) and nuclear genes (Rozas & Aguadé, 1991; Mestres *et al.*, 2004; Gómez-Baldó *et al.*, 2008) and a reduction in allele number and heterozygosity in microsatellite loci (Pascual *et al.*, 2001, 2007). Under the most probable serial introduction scenario, the number of founders in each area was estimated, being notably small for the introduction into South America (i.e. high bottleneck severity index with 7–10 effective founders), but considerably larger for the subsequent introduction into North America (i.e. low bottleneck severity index with around 100–150 effective founders) (Pascual *et al.*, 2007).

In 1981, *D. subobscura* was found for the first time in the Argentinean Andes (San Carlos de Bariloche) (41.15°S, 71.30°W), which mades it likely that flies from Chile crossed the Andes through natural passes (Prevosti *et al.*, 1983). In 1986, *D. subobscura* was collected in large numbers in western Argentina: San Juan (31.54°S, 68.54°W), Mendoza (32.88°S, 68.82°W) and Esquel (42.90°S, 71.32°W) (Prevosti *et al.*, 1989). In 1984, *D. subobscura* was already recorded in Mar del Plata (38.00°S, 57.55°W), on the Atlantic coast of Argentina, although in very low numbers (out of 1300 Drosophila individuals collected, 26 were *D. subobscura*) (López, 1985). Since then further collections in that locality proved that *D. subobscura* was well established there and presented seasonal variation in numbers (Fernández Iriarte & López, 1995). Nevertheless, the colonization of eastern Argentina is poorly understood, and the origin of the founders remains to be tested. Two explanations are possible: either the flies came from Chile across the Andes reaching eastern Argentina, or, alternatively, they came directly from the Palearctic region. According to the first hypothesis, a strong genetic similarity is expected between Argentinean and Chilean (and even North American) populations. If the second hypothesis is correct, the genetic composition would be dramatically different between Argentina and Chile (and North America).

Thus, the aim of the present work was to understand invasion success and track the colonizing history of *D. subobscura* in the eastern coast of Argentina. The main goal was to ascertain whether eastern Argentina was independently colonized from Europe, or, otherwise, is the result of an expansion from the already colonized areas in South America. To answer these questions we analysed data on chromosomal arrangements, lethal genes and microsatellite loci from two eastern Argentinean populations and compared them with previous results obtained for the same markers from Chilean, North American and European populations.

Materials and methods

Sample collection

Drosophila subobscura flies were sampled during the spring of 2005 (November) in Mar del Plata (38.00°S, 57.55°W) and Maipú (36.88°S, 57.85°W). The species was not detected in two more northern localities: Chascomús (35.53°S, 58.02°W) and Monasterio (35.77°S, 57.93°W). Collected females were isolated individually into vials with instant *Drosophila* medium (Carolina Biological Supply Co., Burlington, North Carolina, USA) and their progeny kept for analysis (isofemale lines hereafter). Wild males were kept in groups of 20 individuals in mass cultures with instant *Drosophila* medium until crossed in the laboratory.

Chromosomal inversion polymorphism

Wild males or F_1 males, one from each isofemale line, were individually crossed with four to five virgin females of the strain *chcu,* which is homokaryotipic for the chromosomal arrangements A_{ST}, J_{ST}, U_{ST}, E_{ST} and O_{3+4} and carries the recessive visible markers *cherry* eyes and *curled* wings. Polytene chromosomes from one late third instar female larva per cross were examined using aceto-lacto orcein stains.

Lethal allelism test

To analyse the lethal contents of O_5 inversions in Argentinean populations, we carried out the pattern of crosses described in Mestres *et al.* (1998) using the *Va/Ba* balanced lethal stock. Starting with the F_1 males from the crosses in which an O_5 chromosome was detected, the *Va*/O_5 chromosomal lines obtained were intercrossed to determine whether they carried the same lethal gene (for details, see Mestres *et al.*, 1990). Finally, virgin females of these *Va*/O_5 chromosomal lines were crossed with males of the G7A strain (also *Va*/O_5) from Gilroy (USA), which carries the lethal gene completely associated with the O_5 inversion in all Chilean and North American populations.

Microsatellite variation

Fifty-two individuals were analysed for each population (one F_1 female per isofemale line). Whole genomic DNA was extracted from single-fly squish preparations (Gloor *et al.*, 1993), and microsatellite PCR and allele size identification were conducted according to Pascual *et al.* (2001). The nine microsatellite loci used in this study are a subset of those isolated by Pascual *et al.* (2000b): dsub01, dsub02, dsub04, dsub05, dsub 18, dsub19, dsub20, dsub21 and dsub27. These loci had been previously surveyed in five European (Aarhus, Lille, Montpellier, Barcelona and Málaga), two North American (Bellingham and Fort Bragg) and two Chilean (Puerto Montt and La Serena) populations of *D. subobscura* (Pascual *et al.*, 2001, 2007). These data will be used for comparison in the present work.

Microsatellite data analysis

Genetic diversity in microsatellite loci was estimated by the mean number of alleles per locus, the observed heterozygosity and the expected heterozygosity. Differences between localities were assessed with *t*-tests after normalizing the number of alleles with the square root transformation and the heterozygosity with arcsin transformation. The F_{ST} values and genetic differentiation using Fisher's exact test between the two Argentinean populations as well as deviations from Hardy–Weinberg equilibrium were computed as implemented in GENEPOP version 4 (Rousset, 2008). Pairwise F_{ST} values were also obtained between colonizing populations from different areas.

The origin of *D. subobscura* from Argentina was inferred with a neighbour-joining tree reconstruction using chord distance (Cavalli-Sforza & Edwards, 1967) and comparing populations from Europe (Málaga, Barcelona, Montpellier, Aarhus and Lille), USA (Fort Bragg and Bellingham) (Pascual *et al.*, 2001), Chile (La Serena and Puerto Montt) (Pascual *et al.*, 2007) and Argentina (Mar del Plata and Maipú). Robustness was assessed by 1000 bootstrap replicates over loci using the package POPULATIONS (Langella, 2002). Identification of the most likely source population for Argentinean individuals of *D. subobscura* was also carried out by assignment statistics using the Bayesian computation criteria of Rannala & Mountain (1997) implemented in the software GENECLASS (Piry *et al.*, 2004). The number of chromosomes carried by the sample of colonizers reaching Argentina was estimated by bootstrapping the probability of observing a genetic distance larger or smaller than that empirically observed using as the source of the colonists the most likely population inferred. These simulations, using the program MULTSIM (Noor *et al.*, 2000), allowed the calculation of the maximum and minimum numbers of colonists for each locus. Principal coordinate analysis was carried out with all or only American populations using the package GENALEX 6 (Peakall & Smouse, 2006). The procedure used was based on the standardized Nei's genetic distance.

Results

A total of 590 and 801 *Drosophila* flies were collected in Mar del Plata and Maipú respectively. The most abundant *Drosophila* species was *D. gaucha* (75% in Mar del Plata and 62% in Maipú). *Drosophila subobscura* was the second most frequent *Drosophila* species in both localities, accounting for 19% (65 males and 71 females) and 31% (160 males and 91 females) respectively.

Table 1 lists the frequency of chromosomal arrangements and number of chromosomes sampled (*N*) in the

Table 1 Chromosomal arrangement frequencies and number of chromosomes sampled (*N*) in two natural populations of *Drosophila subobscura* from Argentina and mean chromosomal arrangement frequencies in West South America, North America and Europe.

	Mar del Plata	Maipú	West South America	North America	Europe
A_{ST}	0.446	0.484	0.498	0.568	0.514
A_1	0	0	0	0	0.138
A_2	0.554	0.516	0.502	0.432	0.344
A_{2+6}	0	0	0	0	0.003
$A_{2+3+5+7}$	0	0	0	0	0.002
N	130	155			
J_{ST}	0.346	0.288	0.287	0.375	0.415
J_1	0.654	0.712	0.713	0.625	0.579
J_{3+4}	0	0	0	0	0.006
N	133	163			
U_{ST}	0.394	0.436	0.480	0.455	0.251
U_1	0	0	< 0.001	0.001	0.016
U_2	0	0	0	0	0.003
U_{1+2}	0.295	0.239	0.313	0.325	0.459
U_{1+8}	0	0	0	< 0.001	0
U_{2+13}	0	0	0	0	0.002
U_{1+2+3}	0	0	0	0	0.003
U_{1+2+4}	0	0	0	0	< 0.001
U_{1+2+6}	0	0	0	0	0.016
U_{1+2+7}	0	0	0	0	0.001
U_{1+2+8}	0.311	0.325	0.206	0.219	0.248
N	132	163			
E_{ST}	0.526	0.589	0.579	0.654	0.567
E_8	0	0	0	0	0.024
E_{17}	0	0	0.001	0	0
E_{21}	0	0	0	0.001	0
E_{1+2}	0	0	0.005	0.030	0.143
E_{1+2+9}	0.015	0.037	0.110	0.111	0.074
E_{1+2+22}	0	0	0	0	0.001
E_{1+2+23}	0	0	0	0	< 0.001
$E_{1+2+9+3}$	0.248	0.190	0.180	0.133	0.030
$E_{1+2+9+4}$	0	0	0	0	0.044
$E_{1+2+9+12}$	0.211	0.184	0.124	0.071	0.117
N	133	163			
O_{ST}	0.286	0.215	0.236	0.234	0.405
O_5	0.060	0	0.072	0.058	0.003
O_6	0	0	0	0	0.009
O_7	0	0.012	0.001	< 0.001	0.006
O_{11}	0	0	0	0	0.001
O_{15}	0	0	0	0	0.001
O_{3+4}	0.098	0.129	0.053	0.096	0.198
O_{3+4+1}	0	0	0	0	0.023
O_{3+4+2}	0.346	0.429	0.311	0.313	0.014
O_{3+4+6}	0	0	0	0	0.002
O_{3+4+7}	0.098	0.153	0.126	0.088	0.131
O_{3+4+8}	0.113	0.061	0.202	0.210	0.177
O_{3+4+10}	0	0	0	0	< 0.001
O_{3+4+12}	0	0	0	0	0.003
O_{3+4+13}	0	0	0	0	0.001
O_{3+4+17}	0	0	0	0	0.004
O_{3+4+22}	0	0	0	0	0.011
O_{3+4+23}	0	0	0	0	< 0.001
$O_{3+4+12+13}$	0	0	0	0	< 0.001
$O_{3+4+23+2}$	0	0	0	0	0.003
$O_{3+4+23+6}$	0	0	0	0	0.010
$O_{3+4+2+26}$	0	0	0	< 0.001	0
N	133	163			

The populations used in the comparisons to compute the mean frequencies in the three areas (West South America, North America and Europe) are those listed in Prevosti *et al.* (1988, 1989, 1990) and Balanyà *et al.* (2003, 2006).

natural populations of *D. subobscura* from Mar del Plata and Maipú. A total of 18 chromosomal arrangements were detected, all of them present in Chile and North America (Prevosti *et al.*, 1988, 1989, 1990; Balanyà *et al.*, 2003). If we exclude the inversion E_{17}, newly formed in South America and detected only in two samples taken in 1981, only two of the arrangements present in Chile are absent in the eastern Argentinean samples: E_{1+2}, detected only in five of the nine Chilean populations previously analysed and at a frequency always lower than 4%, and U_1, detected only in Chillán and derived most probably from a rare recombination event between U_{1+2} and U_{ST} (Balanyà *et al.*, 2003). Interestingly, although in Chile the O_5 arrangement is present in all populations with a frequency ranging from 1.5% to 14.2%, this arrangement was detected only in the sample from Mar del Plata (Table 1). The O_7 arrangement, detected only in Maipú at a frequency of 1% (Table 1), is present in four Chilean populations in the range of 0.3–0.7%. This arrangement probably derives from a rare recombination event between O_{3+4+7} and O_{ST} (Sperlich & Feuerbach-Mravlag, 1974; Balanyà *et al.*, 2003; Gómez-Baldó *et al.*, 2008; Mestres *et al.*, 2008). Similarly, besides E_{1+2}, the only inversions present in North America not detected in Argentina are the newly formed inversions E_{21} and O_{26} (Table 1). As the microsatellite loci point to the Chilean localities as the most probable source of the colonizers of Argentina (see below), the F_{ST} values between both Argentinean populations as well as with two Chilean populations have been estimated (Table 2). The smallest F_{ST} value corresponds to the comparison between both Argentinean localities.

All O_5 inversions extracted from Mar del Plata (lines MDP35, MDP59, MDP63 and MDP115) proved to carry

Table 2 F_{ST} values between Argentinean and Chilean populations estimated using chromosomal arrangements and microsatellite loci.

Chromosomal arrangements		Microsatellites	
Mar del Plata – Maipú	0.0037	Mar del Plata – Maipú	0.0010
Mar del Plata – Puerto Montt	0.0046	Mar del Plata – Puerto Montt	0.0153
Mar del Plata – Santiago	0.0079	Mar del Plata – La Serena	0.0094
Maipú – Puerto Montt	0.0058	Maipú – Puerto Montt	0.0164
Maipú – Santiago	0.0062	Maipú – La Serena	0.0050

at least one lethal gene. The genetic crosses among them demonstrated that these four O_5 chromosomal lines carried the same lethal gene. Virgin *Va*/O_5 females obtained from each of these lines were also crossed with *Va*/O_5 males of the lethal chromosomal line G7A from Gilroy (USA). All crosses confirmed that the lethal gene present in the O_5 arrangements from Argentina (Mar del Plata) was allelic with that found in Gilroy. Thus, the same complete association between a lethal gene and the O_5 inversion found in Chile and North America (Mestres *et al.*, 1992, 1995, 2005, 2008; Solé *et al.*, 2000) is also detected in Argentina.

The number of alleles per locus (k) and the expected mean heterozygosity (H_e) at the nine microsatellite loci analysed were similar in Mar del Plata and Maipú, and the observed mean heterozygosity (H_o) was smaller than H_e for both populations. However, both populations did not deviate from Hardy–Weinberg expectations ($\chi^2_{18} = 20.37$, $P = 0.312$, $\chi^2_{18} = 18.02$, $P = 0.454$ respectively). The mean number of alleles per locus and the expected mean heterozygosities were compared between Argentinean, North American, Chilean and European populations (Table 3 and Supporting Information Appendix S1). Argentinean populations presented significantly smaller number of alleles ($t_5 = 8.08$, $P = 0.0005$) and expected heterozygosity ($t_5 = 9.47$, $P = 0.0002$) than European populations. When Argentinean populations were compared with the other American populations a significant reduction in the number of alleles was also observed ($t_4 = 5.02$, $P = 0.0074$), although no differences in heterozygosity were detected ($t_4 = 1.17$, $P = 0.3063$). Without exception, all alleles found in Argentina were also detected in Chilean populations. However, rare alleles found in Chile with a frequency lower than 0.05 were not found in Argentina, with the exception of allele 248 from locus dsub02 that was present in both Argentinean samples in spite of having a smaller frequency in all South American populations and not being detected in North American populations (Appendix S1).

Table 3 Mean number of alleles per locus (A) and expected mean heterozygosity (H_e) for microsatellite loci in colonizing (America) and endemic (Europe) populations.

Continent	Country	Population	A	H_e
America	USA	Bellingham	5.667	0.685
		Fort Bragg	5.222	0.726
	Chile	Puerto Montt	5.556	0.744
		La Serena	5.444	0.702
	Argentina	Mar del Plata	4.778	0.690
		Maipú	4.778	0.703
Europe	Denmark	Aarhus	11.889	0.839
	France	Lille	14.333	0.871
		Montpellier	15.667	0.875
	Spain	Barcelona	17.222	0.887
		Málaga	16.667	0.893

No genetic differentiation was observed for the microsatellite loci between Mar del Plata and Maipú ($\chi^2_{18} = 25.93$, $P = 0.101$) in accordance with the small F_{ST} value between them (Table 2). As populations within Chile and North America were not significantly differentiated (Pascual *et al.*, 2007), data from each region were pooled for analyzing genetic differentiation between them as well as for tracing the origin and effective number of the Argentinean colonizers. All comparisons between Argentinean populations and the other population regions revealed significant genetic differentiation for all of them ($P < 0.00001$), with smaller F_{ST} values when compared with Chilean populations ($F_{ST} = 0.010$) than with North American populations ($F_{ST} = 0.034$) or European populations ($F_{ST} = 0.099$).

The unrooted neighbour-joining tree based on chord distance clearly separates the European from the American populations (Fig. 1). The American colonizing populations split into two branches with nodes associated

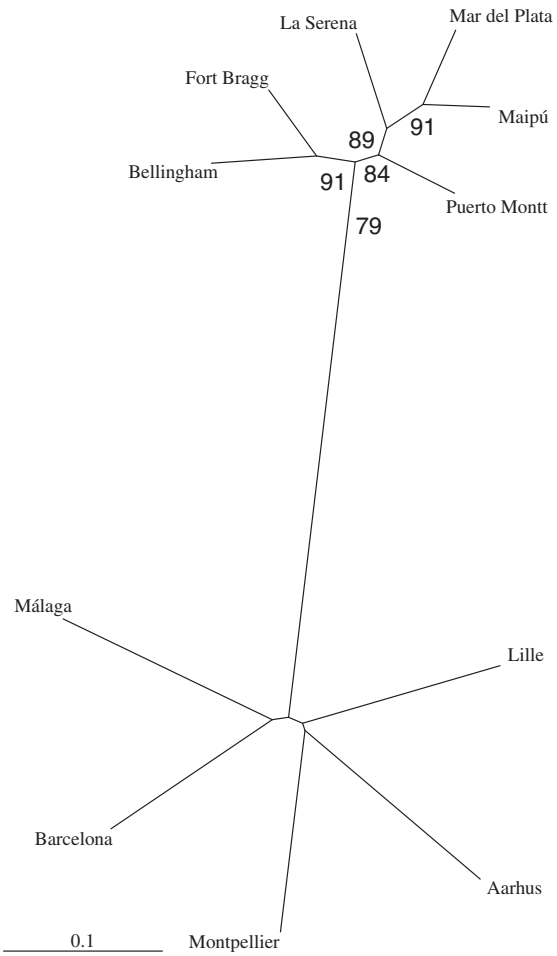

Fig. 1 Unrooted neighbour-joining tree relating ancestral (European) and colonizing (American) populations of *Drosophila subobscura*. Bootstrap values higher than 60% are given.

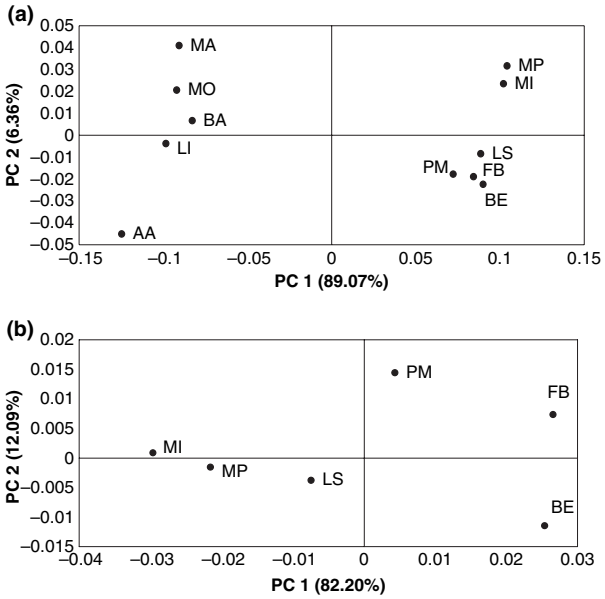

Fig. 2 Multilocus principal coordinate analysis for: (a) ancestral and colonizing populations, (b) only colonizing populations of *Drosophila subobscura*. BE, Bellingham; FB, Fort Bragg; PM, Puerto Montt; LS, La Serena; MP, Mar del Plata; MI, Maipú; AA, Aarhus; LI, Lille; MO, Montpellier; BA, Barcelona; MA, Málaga.

with high bootstrap values: one includes North American populations whereas the other clusters Chilean and Argentinean populations (Fig. 1). A principal coordinate analysis based on Nei's genetic distance separates European from American populations by the first axis explaining 89% of the variation (Fig. 2a). When only colonizing populations are considered (Fig. 2b), the Chilean populations are centrally located on the first axis, which explains 82% of the variation. Furthermore, when tracing the origin of the colonizers by Bayesian inference, the Argentinean populations were assigned to the Chilean populations with 100% probability. All these lines of evidence corroborate that the Argentinean *D. subobscura* founders came from Chile and it is very unlikely that they independently came from Europe or have a North American origin.

The minimum and maximum number per locus of Argentinean colonizers was estimated using Chile as the most likely source after pooling the data within each area. The mean number of colonizers, averaging only autosomal loci, was 110 and 150 individuals for the minimum and maximum, respectively, and 80–120 when averaging only X-linked loci after correction for chromosome number.

Discussion

An invasive species able to reach a new region in a recurrent way is expected to show high levels of genetic diversity facilitating its adaptation to the new habitat. However, this is not the only way to succeed in a new invasion: a low number of founders carrying the appropriate genetic variability could be able to successfully establish in a new area. A stable colonization is most feasible if the environmental conditions of the new habitat are very similar to those found in the original area of distribution of the species because natural selection, among other evolutionary forces, would act from the beginning conditioning the success or failure of the invasion.

These conditions seem to have occurred in the colonization of America by *D. subobscura*. It represents a unique event of colonization, with only one big primary bottleneck. It has been shown that all the American colonizing populations are genetically related (Prevosti *et al.*, 1988; Mestres *et al.*, 2005; Pascual *et al.*, 2007) including the Argentinean populations analysed in the present work. Nonetheless the species in America has not lost its potential for adaptation (Balanyà *et al.*, 2003, 2006). In North America and Chile the species settled down where conditions similar to those in its native areas were found (Ayala *et al.*, 1989; Prevosti *et al.*, 1989). The colonized area of the Atlantic coast of Buenos Aires Province, where the populations analysed in the present study are located, is mainly agricultural, with isolated pockets of forests composed mostly by nonautochthonous trees. The climate in this area is oceanic and similar to that found in southern Chile and certain regions of Europe. As natural selection has been shown to act on the chromosomal polymorphism of this species (Balanyà *et al.*, 2006), we would expect that the populations located in areas with similar climatic conditions would present a more similar chromosomal polymorphism. In agreement with this expectation, the chromosomal polymorphism of the Argentinean populations is more similar to that in Puerto Montt (southern Chile) than in Santiago (Table 2).

In colonized populations from Argentina, a total of 18 chromosomal arrangements have been detected, out of the approximately 80 recorded in the Palearctic region. The arrangements observed are, in general, those most frequent in the Old World (Balanyà *et al.*, 2003). Chromosomal arrangements that are uncommon in Europe (i.e. O_{3+4+2} and O_5) but present in the colonizing populations of Chile and USA are also detected in the colonizing populations of Argentina. This is in agreement with the hypothesis that these populations do not represent a new colonization event from Europe. Moreover, all the O_5 arrangements from Mar del Plata proved to carry the same lethal allele completely associated with the Chilean and North American O_5 inversions (Mestres *et al.*, 1990, 1992, 1995, 2005, 2008; Solé *et al.*, 2000). Therefore both lines of evidence, i.e. chromosomal arrangements and lethal genes, conflate to show that the American colonizing populations (Chile, USA and Argentina) derive from the same event of colonization.

The tenet that Argentina was not independently colonized from Europe is reinforced by the fact that the observed alleles of the nine microsatellite loci analysed are almost similar to those in all the other American populations (Appendix S1). A rare allele found only in Chilean populations was also observed in the Argentinean ones supporting the hypothesis of those populations being the source of the Argentinean populations. This hypothesis is further bolstered by the neighbour-joining tree that shows that the populations from Argentina are clustered with the populations from the western colonized areas of South America, from which they probably originated (Fig. 1). Furthermore, the assignment tests unambiguously identified the Chilean populations as the source of the Argentinean ones.

The number of microsatellite alleles observed in Argentina was slightly, but significantly, lower than in the other two colonized areas (Table 2), indicative of a secondary founding event during the colonization of Argentina. However, no significant differences were observed when comparing the expected heterozygosity among these three regions in agreement with theoretical and experimental studies of population bottlenecks, which indicate a larger impact in the number of alleles per locus than in heterozygosity between pre- and post-bottleneck populations (Nei *et al.*, 1975; Balanyà *et al.*, 1994; Pascual *et al.*, 2001). This reduction in allele number is probably responsible for the greater similarity of both Argentinean populations with La Serena (Table 2) which can be considered a marginal population, having a more reduced microsatellite variability in comparison with Puerto Montt (Pascual *et al.*, 2007). The mean inferred number of founding individuals from Chile ranges between 80 and 150 depending on whether the loci are in the sex chromosome or the autosomes. This is in agreement with the X-chromosome being more sensitive to bottleneck detection even from a large sample of colonizers (Pool & Nielsen, 2007). These values on the number of colonizers are slightly smaller than those inferred between Chile and USA as the minimum (45) and maximum (245) number of colonizers (Pascual *et al.*, 2007). This indicates that the founder event was probably stronger when expanding to Argentina. Nevertheless we cannot rule out an eastward expansion by a much larger number of individuals with multiple steps and bottlenecks (Noor *et al.*, 2000). Further sampling of populations on a west–east transect should be carried out to discriminate between these two possible scenarios. Our data are also compatible with multiple and recurrent invasions of the east coast of Argentina. However, the detection of a secondary bottleneck would indicate that the number of introductions would be low.

In summary, altogether this evidence strongly suggests that Argentina was not independently colonized by *D. subobscura* from the Palearctic region, but that it was rather invaded by a rapid population expansion from Chile with a light secondary bottleneck. This indicates that invasive species, in spite of dramatically reducing their genetic variability after a founder event, do not lose their potential to establish in new environments provided that ecological conditions are adequate. The methods used in this work prove useful to track the origin of new invasions and to ascertain whether they constitute independent or secondary colonizations. As the number of invasive species is expected to grow with climate change and global trade increase, knowledge of the invasive histories can help to develop managing strategies and prevent future invasions.

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Supporting information

Additional supporting information may be found in the online version of this article:
Appendix S1 Number of *Drosophila subobscura* individuals (*n*) and allele frequencies for each of the nine microsatellite loci genotyped in Mar del Plata and Maipú (Argentina). The mean number of genotyped individuals per population and mean allele frequencies of two Chilean, two North American and five European populations are given for comparison (Pascual *et al*., 2007).

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