ISSN 0373-580 X
Bol. Soc. Argent. Bot. 45 (3-4): 333-342. 2010

GENE FLOW AND LOCAL ADAPTATION: ANTAGONISTIC FORCES SHAPE POPULATIONS OF *ILEX DUMOSA* (AQUIFOLIACEAE)

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Summary: The evolutionary forces acting in a set of populations may vary in space, resulting in Genotype x Environment interaction for Darwinian fitness, fixing in some populations adaptative traits of a local population to a specific environmental conditions or, by gene flow homogenize the genes frequencies and make a set of population adapted to a group of environments. This paper tries to elucidate the population dynamics of a set of populations of *Ilex dumosa* Reiss., in which was measured quantitative traits of six populations from six geographic regions and test their responses to environmental changes. The experimental approach was made in common garden experiment and by reciprocal transplant experiment. Local adaptation was investigated both by native superiority over non-natives, and by comparing the observed performance of a population to the fitted value of a reduced statistical model that showed the populations' performance at all sites and the performance of all other populations at its home site. Were found evidence of a core population in the center of the distribution area, homogenized by gene flow, and one marginal population with patterns of local adaptation, evidenced the with highest native superiority in his environment, and the worst behavior in the other environments.

Key words: Darwinian fitness, genotype x environment interaction, marginal speciation, reciprocal transplant experiment, stabilizing selection.

Resumen: Flujo génico y adaptación local: Fuerzas antagónicas modelan *Ilex dumosa* (Aquifoliaceae). Las fuerzas evolutivas que actúan en un conjunto de poblaciones pueden variar en el espacio, resultando en patrones de interacción Genotipo x Ambiente para la aptitud reproductiva, fijando en algunas poblaciones caracteres adaptativos para una población local a un conjunto de condiciones ambientales especificas o, por flujo génico homogenizar las frecuencias génicas y tener un conjunto de poblaciones adaptadas a un grupo de ambientes. Este trabajo trata de dilucidar la dinámica poblacional de un grupo de poblaciones de *Ilex dumosa* Reiss. en el cual se midieron caracteres cuantitativos de 6 poblaciones de 6 regiones geográficas y se estudiaron las respuestas a cambios ambientales. Para llevar a cabo dichos estudios se realizaron experimentos en jardín común y de trasplante reciproco. La adaptación local fue puesta a prueba mediante un enfoque de nativas vs. no-nativas, y comparando el rendimiento observado de las poblaciones a un valor fijo de un modelo estadístico reducido que muestra el rendimiento de todas las poblaciones en todos los sitios y de todas las poblaciones en sus sitios originales. Fue encontrado un grupo de poblaciones núcleo, en el centro del área de distribución, homogeneizada por flujo génico, y una población que presenta patrones de adaptación local, demostrado por poseer el mayor rendimiento (en términos de aptitud darwiniana) y la peor en los demás ambientes.

INTRODUCTION

The forces of natural selection often vary in space, resulting in genotype x environment interaction for Darwinian fitness. In absence of other forces, divergent selection could favor in each local population (deme) the presence of traits that provide an advantage under its local environmental conditions (Kawecki & Ebert, 2004). Thus, under natural selection, individuals tend to adapt to their local environmental conditions, resulting in patterns of local adaptation (Lenormand, 2002). The evolution of locally adapted genotypes requires consistent geographic variation in selective regimes that cause directional trait changes, as well as limited gene flow among populations. Differences in selection pressures may lead to specialized local

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ecotypes due to heterogeneity in abiotic factors like climate (Joshi *et al*., 2001; McKay *et al*., 2002) and soil conditions (Snaydon & Davies, 1982; Gauthier *et al*., 1998). However, the differences in selection pressures can also be result of different biotic factors like competitors, parasites, pathogens or mutualists (Parker, 1995; Linhart & Grant, 1996; Prati & Schmid, 2000; Gilbert, 2002). Because plants are fixed, typically have limited gene dispersion through seeds and pollen, they experience generations of selection in their local environmental conditions, this way its enhanced the performance of each deme in his environmental condition (Galloway & Fenster, 2000). The degree of local adaptation depends on a balance between local selective pressures and the dispersal processes. Gene flow can constrain adaptation to a spatially heterogeneous environment by preventing local differentiation (Slatkin, 1987). Therefore, a negative association is expected between dispersal processes and local adaptation. Only very strong selection can maintain local adaptation against a substantial gene flow.

The distribution area of *Ilex dumosa* Reiss. presents a moderate environmental heterogeneity, in both climatic and soil composition, furthermore its critically disturbed by mankind (Giberti, 1999), leading the habitat fragmentation in this area. This habitat fragmentation, which results in decreased population size and increased isolation of populations, may influence the extent of local adaptation. However, the effects of this habitat fragmentation can be difficult to predict. On one hand, fragmentation might be expected to increase local adaptation, because gene flow is lower among isolated populations. On the other hand, the effects of random genetic drift in small populations would become more important than those of selection, reducing or eliminating existing local adaptations (Frankham *et al*., 2002)

Studies of local adaptation have been for a long time an important part of the study of evolution (Turesson, 1922; Clausen *et al*., 1940). A common way to examine local adaptation is to make reciprocal transplantation experiments (Waser & Price, 1985; Nagy & Rice, 1997; Kawecki & Ebert, 2004), where better performance of natives compared to non-natives indicates local adaptation. Kawecki and Ebert (2004) distinguish between two different criteria proposed to detect local adaptation in such experiments. First, the ‘‘local vs. foreign’’ criterion, meaning that native individuals on average should be superior to non-natives, second, the ‘‘home vs. away’’ criterion, meaning that individuals on average should perform better in their native habitat compared to foreign habitats. These two criteria may be simultaneously satisfied indicating local adaptation (Thrall *et al*., 2002; Belotte *et al*., 2003).

Moreover, under environmental conditions at a transplant site, genetic differences between populations can be studied by quantifying the phenotypic differences among plants of different origins, and examine the responses to different environments (Linhart & Grant, 1996; Briggs & Walters, 1997).

Ilex dumosa, an adulterant of the yerba mate (*Ilex paraguariensis* A. St.- Hil.*)* that have a widespread distribution in South America (Fig. 1), including northwestern Uruguay, southeastern Brazil, northeast of Argentina and Paraguay (Giberti, 1989). It is a shrub or small tree 1-8 meters tall, dioecious, with solitary flowers, evergreen, with crossed pollination and flowering all the year, pollinated by bees and other insects. Seeds are dispersed by birds, which are the main means of dispersion of this species (Giberti, 1999).

Analyzing 32 genotypes from six localities, which represents extremes and mid populations in the distribution area. Through quantitative traits in a common garden and reciprocal transplantation experiments (RTE), was attempted demonstrate the presence of genetic differences among the genotypes and the influence of the environment on these genotypes in response to a change in their local conditions by a relative performance analysis, of each genotype in each environment.

This study examines the population differentiation and local adaptation in some populations of *I. dumosa*, exploring home site advantages of native populations compared to non-native populations both in terms of native superiority and in terms of relative performance.

MATERIAL AND METHODS

Biological material

For this study, 32 genotypes from six localities (Table 1) with different local environment were used. They were well represented in each locality and distanced among them with a mean distance of

Fig. 1: Distribution area of *Ilex dumosa* in the elliptic zone. Sampled populations: 1. Oberá, 2. Campo Viera, 3. Clevelandia, 4. Caçador, 5. Palmas, 6. Campo Bom.

80 km, being the locality 6 distanced from the others by more than 160 km. These genotypes consist of juveniles with 2 to 4 leafs of 8 to 10 cm tall, obtained in a greenhouse since germination at 12 months. Greenhouse condition was a composed medium with 50% of subsurface soil poor in organic matter and 50% of sand. The disinfection of the same was done with hot water (80-90ºC). Disease prevention was made with benomyl and methyl tiofanto in rotation. The juveniles were ready for the experiments in February 2004.

Common garden measurements: differentiation among populations

To study if the differentiation among the populations has a genetic component, the genotypes were grown in a common garden. The experimental design was totally randomized with 960 plants, in a surface of 9 ha, represented the 32 genotypes, in 3 repetitions with 10 plants/genotype/repetition. This study was performed in the experimental station INTA Cerro Azul, Misiones, Argentina, placed approximately in the center of the distribution area of the species (27º39´13,68´´ S, 55º26´11,28´´ W), characterized by soils with high content of iron and aluminum. The climate is humid subtropical (hot summers), with temperatures in the colder months between 0 and 18°C, in the warmest months with average temperatures above 32ºC and an average annual rainfall of 1600 mm. After a year (March, 2005) were measured the plant height, number of flowers both male and female and biomass production, the aerial parts of the plants were dried in a stove for 24 hours at 85ºC, subsequently were weighted, this method was make in each individual. The genetic differentiation was analyzed as function of fixed region.

Transplantation experiment

The same genotypes tested in common garden were transplanted in May 2005 as juveniles near of the original location where the samples were collected, this is it Campo Viera and Obera (Argentina), Caçador, Clevelandia, Palmas and Campo Bom (Brazil) in a surface similar to the utilized in the common garden experiments. The experimental design was a randomized block design, with 3 blocks, inside the blocks were placed plots, containing the 32 genotypes, and each plant was repeated 10 times, the environmental conditions at each experimental field is detailed in Table 1. After one year (April 2006), the same traits of the common garden experiment and the proportion of survival were measured.

Home vs. Away analysis

To investigate if natives perform better than non-natives, the traits proportion of survival plants, the number of flowers, plant height and biomass production were analyzed with a full model including the interaction term between genotypes x sites. When there were evidences of interaction, a comparison between natives vs. non–natives plants was done.

Moreover, the distance between the populations were estimated by the multivariate Penrose distance method, with all genotypes and all traits measured. In addition, populations were grouped by clustering methods with complete linkage criteria. To group the populations in relation of the average fitness given by the genotypes present in each of them.

Analysis by relative performance

To analyze the relative difference in performance for all native populations was used the same data as in the Home vs. Away analysis. In the full factorial linear statistical models used in this study

$$Y_{ijk}= \mu...+ \alpha_i+ \beta_j+ (\alpha\beta)_{ij}+ \varepsilon_{ijk}$$

the fitted values are equal to the treatment means $Y_{ijk} = Y_{ij}$:. In the reduced models were omitted the interaction term $(\alpha\beta)_{ij}$,

$$Y_{ijk}= \mu...+ \alpha_i+ \beta_j+ \varepsilon_{ijk}$$

it can be shown that the fitted values of the means are equal to $Y_{ijk} = Y_{.i:} + Y_{:j:} - Y_{...}$ (Neter *et al.*, 1996). If is compare the observed average value for a population at its home site to the predicted value from a reduced model. Will be able to tell if the population is performing better or worse than predicted from its own overall performance at all sites ($Y_{.i.}$), and from the mean performance of all other populations at its home site ($Y_{:j:}$) in relation to the mean performance of all populations at all sites ($Y_{...}$). In this way is estimating the adaptation to a specific site irrespective of site quality and original differences among populations (Jakobsson & Dinnetz, 2005). Were used the fitted values from the reduced models from each transplantation group to see if the 6 native populations performed better than predicted at their home sites. The predicted mean and the observed mean from each population were then treated as paired independent observations. To test for a general pattern of local adaptation across all six populations was analyzed the difference between observed and predicted values in a paired test for dependent samples for continuous variables and in Wilcoxon's matched pair test for qualitative variables. With this method was investigated local adaptation by relative performance in the following response variables: survival proportion, flower number, plant high and biomass.

All variables were analyzed using BioEstat5.0.

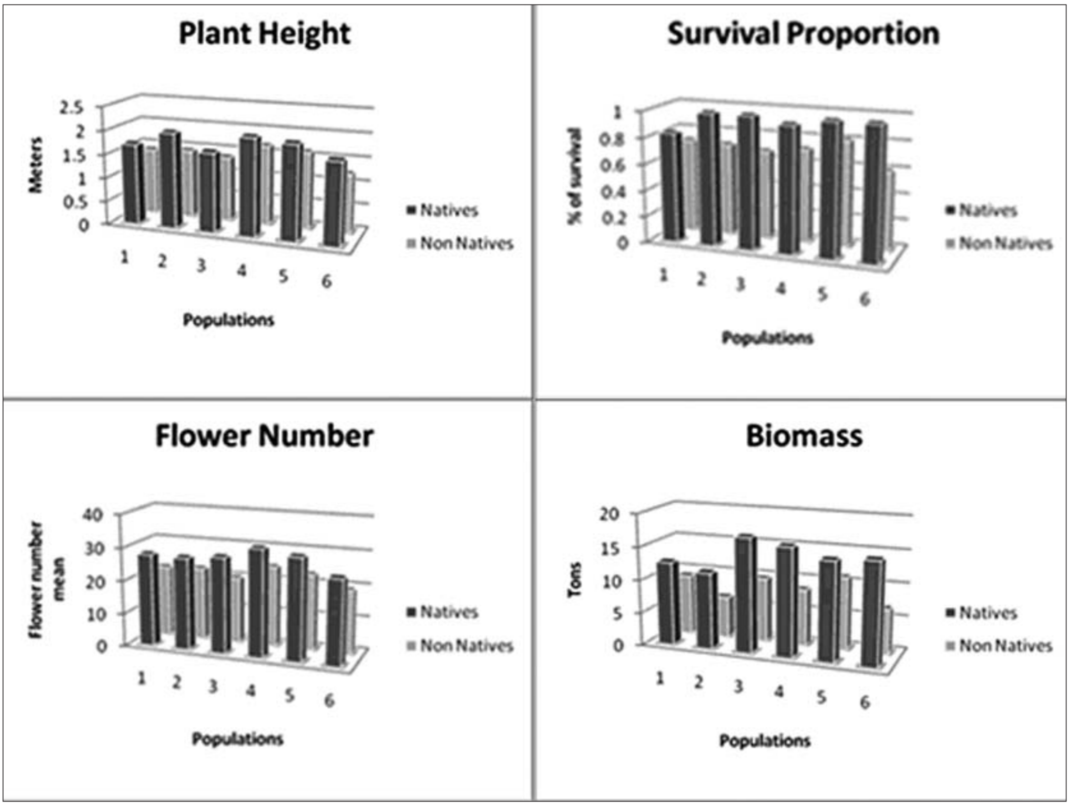

Fig. 2: Performance of natives vs. foreign populations for each trait measured.

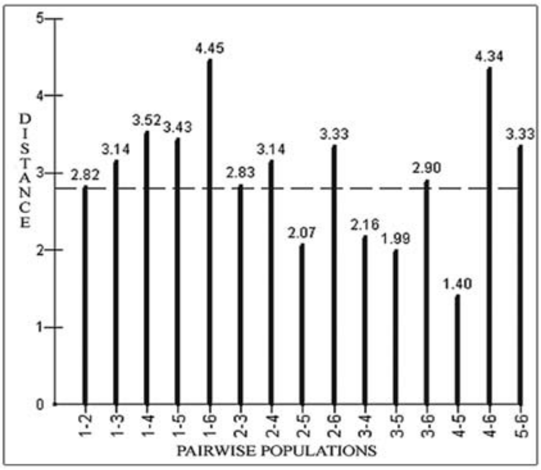

Fig. 3: Distance among the populations with all the traits analized, the population 6 have the biggest distance in relation to the others populations.

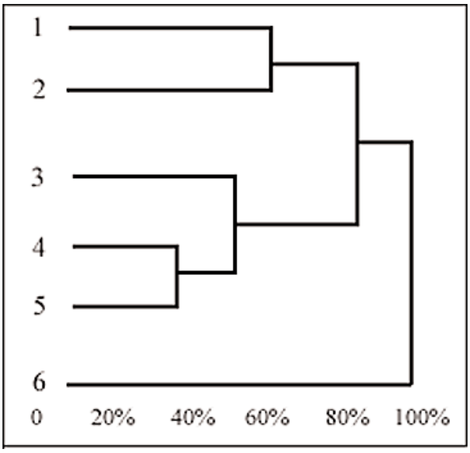

Fig. 4. Population clustering through the survival proportion, show the grouping of population more nerby and the population 6 alone, being the more away from the others.

RESULTS

Differentiation among populations

Through the common garden experiment was evident the presence of significant differences between genotypes and among regions in the biomass production, and plant height and Flower number only show significant differences between genotypes (Table 2).

Home vs. Away

The results of the reciprocal transplant experiment (RTE) are similar to the results of the common garden experiments (Table 3), and shown the role of the environment in the behavior of the traits. Moreover, each genotype presented a superior performance for each trait in his respective environments (Fig. 2).

The Euclidean distances between the core populations (populations 1 to 5) and the population 6 are above the mean, respect to all distances between all pairwise of populations as shown in the Figure 3. This is consistent with the pattern shown in Figure 4, where the population 6 is separated from the other populations by 100% of Euclidian distance. Thus, the genotypes belonging to the population 6 could show patterns of local adaptation.

Native superiority

All the traits in transplantation groups show a significant genotype x environment interaction (Table 3). In this experiment, Survival Proportion has shown the major genotype component, in contrast the Biomass Production show a contribution of the environment, although small in magnitude, highly significant. Plant Height and Flower number have a similar behavior, which is typical of quantitative traits.

The relative performance test shows that only the survival proportion have significant differences between the observed and expected mean (Table 4), according to the fact that genotypes in the different environments show the same behavior, except in the region 6, in this region the natives genotypes have high SP, but his SP is the less in the other regions.

DISCUSSION

With the results of the common garden experiment, the genetic differences among the populations were in evidence, here can be assumed that the predominant component of the variance in the Biomass production is the environmental component, because the differentiation it´s mainly between the populations. However, the other traits analyzed show a major differentiation among the populations, it´s mean the predominant component of the variance is the genotypic.

The existence of such differentiation is a pre req-

Table 1: Origins of the genotypes, environmental conditions and GPS localization (Source: EMBRAPA, Secretaría de Agricultura, Ganadería y Pesca de la República Argentina). Ar= Argentina, Br= Brazil.

Genotypes	Populations	Province/State	Country	Weather/Tº Max y Min/RP	Soils Types	Location
1-juli	Campo Viera	Misiones	Ar	Subtropical/0-40ºC/ 1600 mm year-1	Rich in Fe and Al	27º23´29´´S 55º02´36´´W
agos-13	Oberá	Misiones	Ar	Subtropical/0-40ºC/ 1600 mm year-1	Rich in Fe and Al	27º29´1´´S 55º08´16´´W
14-20	Caçador	Sta. Catalina	Br	Subtropical/13-25ºC/ 180- 1000 mm year-1	Rich in Fe and P	26º43'31"S 51º00'54"W
21-22	Clevelandia	Paraná	Br	Subtropical/10.5-19ºC/ 2051 mm year-1	Rich in P and Al	26º00´23´´S 52º48´00´´W
23-25	Palmas	Paraná	Br	Subtropical/11.6-19.5ºC 2120 mm year-1	Rich in P and Al	26º29´3´´S 51º59´26´´W
26-32	Campo Bom	Río Grande do Sul	Br	Subtropical/14.5-40ºC 400 - 900 mm year-1	Sandy, rich in P and Fe	29º38´18´´S 51º05´33´´W

Table 2. Variation among six populations of *Ilex dumosa* grown in a common garden experiment one year after pruning shape analyzed among populations, and between regions in a general linear model. Genotypes and regions were treated as random factor. ns= p>0.05; *= p< 0.05; **= p< 0.025; ***= p< 0.005.

Variable	Source	df	MS	F	p
Biomass	Region	5	54920848,24	14.5	**
	Genotype	31	41355290,16	10.7	***
	Error	54	8993456,804	14	*
Flower Nº	Region	5	8.821	7.15	*
	Genotype	31	115.518	12.25	***
	Error	54	9.927	5.1	*
Plant Height	Region	5	0.07	8.88	*
	Genotype	31	0.454	15.024	***

Table 3. Variation among the genotypes in reciprocal transplantation experiment, evaluated with the Genotype x Interaction component, where % it´s the proportion of variation explained by the correspondent source, and SP is the Survival Proportion of the juveniles at a year before they were transplanted. ns= p> 0.05; * = p< 0.05; **= p< 0.025; ***=p< 0.005.

Variable	Source	Df	MS	F	p	%
SP	Genotype	31	6.73	5.5	***	24.16
	Environment	50	0.755	7.1	*	2.71
	GEI	9	19.089	5.3	***	68.44
Plant Height	Genotype	31	12.049	4.2	**	17.65
	Environment	50	1.977	17.84	***	2.89
	GEI	9	36.708	4.78	***	53.78
Flower Nº	Genotype	31	1.815.942	7.26	***	10.06
	Environment	50	614.293	12.01	**	3.4
	GEI	9	10.866.096	4.88	***	60.2
Biomass	Genotype	31	1.370.466.362,03	5.48	***	12.52
	Environment	50	1.019.535.763,18	20.39	***	9.31
	GEI	9	6.818.032.896,49	4.63	**	62.29

Table 4. Adaptation by relative performance investigated in six populations of *Ilex dumosa.* The table shows the results from paired tests between observed and predicted values for Survival Proportion (SP), Flower Number (FN), Plant Height (PH) and Biomass (B). Using paired t-test for quantitative variables and Wilcoxon's matched-pair test for proportions. n.s=p>0.05.

Trait	SP		FN		PH		B	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
Mean	0.8217	0.73	1.726	1.712	1.991	24.569	10380,974	110380,324
SD	0.244	0.233	0.382	0.381	6.216	5.544	4840,992	4901
t (paired test)			1.93		0.24		12.3	
Wilcoxon z	0.019							
P			ns		ns		ns	
N	960	960	960	960	960	960	960	960

Table 5. Local adaptation by native superiority in the six population of *Ilex dumosa*, tested by Chi-square in the trait Survival Proportion. ns= p>0.9; *=p<0.9; **=p< 0.05.

Variable	Source	Df	Chi-square	P
SP	Region	3	8	**
	Population	3	3.18	ns
	GxE	31	18.33	*

uisite for local adaptation (Kawecki & Ebert, 2004). This differentiation may be attributed to the spatial heterogeneity, which facilitates the maintenance of polymorphisms, providing substrate for the operation of the density dependence selection in each deme, within demes (Levene, 1953).

Density dependence selection operates independently in different demes favors rare alleles that improve fitness in a habitat, in which most individuals perform poorly. This is a form of frequency-dependent selection, which helps to maintain polymorphism, even when the average fitness of heterozygote is below of both homozygotes (Dempster, 1955).

Protected polymorphism in a heterogeneous environment could be maintained even if dispersal results in a complete mixing of the gene pool. However, in such case demes will not differentiate genetically, i.e. there will not be local adaptation. Thus restrict gene flow, is a pre-requisite for local adaptation. Restricted gene flow also makes a condition for maintenance of polymorphism more favorable (Maynard Smith, 1966).

Another pre-requisite for local adaptation is the presence of Genotype x Environment interaction for the fitness. The results show the amount of GEI that make clear the dependence of the genotypes to their environmental conditions, (in the traits analyzed). Above all, the Biomass production, in which the GEI component explain more than 60% of the variance, these patterns are repeated in the Number of Flowers and Plant Height, but Survival Proportion although have a great environmental and interaction component, the genotypic component it's the major above all the analysis traits.

The achieving of this requisite it´s not rare, because Genotype x Environment interactions is commonly observed in many quantitative traits (Comstock & Moll, 1963; Gupta & Lewontin, 1982; van Tienderen & van der Toorn, 1991) including fitness components (Dobzhansky, 1955; Tachida & Mukai, 1985). This would seem to provide a natural explanation for the maintenance of quantitative variation among populations, favoring the presence of polymorphism in each deme.

Although that pre requisite were achieved, in most of the populations was not detected local adaptation with the away vs. home criteria (Kawecki & Ebert, 2004). This may suggest the genes frequencies could be homogenized in the populations, as a metapopulation model (without cycles of extinction and colonization, only as a model in which are grouped few populations), resulting in patterns of gene flow, with a homogenizing effect preventing local adaptation.

This may be due to some assumptions makes for Slatkin (1973) in which suggest the existence of a characteristic length scale of variation, Z, which determines the equilibrium pattern of gene frequencies in a spatially distributed population. If the environment changes on a scale less than the characteristic length, then the gene frequencies will not respond to the local variations but they will respond to the selection intensities over that characteristic length. When the scale of variation in the environment is larger than the characteristic length, the gene frequencies can respond to the local variations resulting in genetically differentiated populations with clines between them. The result does not depend on the exact form for the dispersal function, M, only on the average distance between the initial and the final locations of an individual.

Under that assumption, the genotypes from the population 6 are adapted to his local habitat and less adapted to the other habitats. The others genotypes shown less performance in the region 6, consequently, this is a marginal region in the distribution of *I. dumosa* separated from the other populations (more than 160 Km) with a distance major of the characteristic length avoiding the homogenizing effect of the gene flow present in the core populations. In addition, the hypothesis that local adaptation is more likely when populations are native of more divergent environments was supported by the greater frequency of home site advantage in comparisons with populations native to different habitats than between populations from the same habitat (Figure 2). The results of several studies that, like this one, have included a range of environmental differences among transplant sites (Figure 3 and 4) are also consistent with greater evidence of local adaptation for populations from more divergent environments.

However, adaptation to new habitats posits few problems. On one hand, marginal populations tend to be demographic sinks (Dias, 1996). Since the population is initially not adapted to the marginal habitat, reproductive success is low and immigration may be necessary in order to maintain the local population. On the other hand, the immigrants bring along genes that, in their evolutionary history, were mostly exposed to natural selection in the core habitat. These problems can be solved, by an assumption that explain the relationship existent between the dispersal rate and the conditions for invasion of a rare allele that improves fitness in a marginal habitat (sink) but reduces fitness in the core habitat (source) (Felsenstein, 1976; Hedrick *et al*.,1976).

This relationship is not generally monotonic: its sign and shape depend on the magnitude of the effect of the rare allele. If the allele reduces fitness in the main habitat only slightly, the condition for its spread is most favorable at the maximum dispersal rate and becomes increasingly stringent as the dispersal rate decreases. Conversely, if the effect of the allele is large enough to reverse the source and sink roles between the habitats, the condition becomes increasingly favorable as the dispersal rate decreases.

For a broad range of alleles with intermediate effects, the condition for increase when rare is most stringent at an intermediate dispersal rate. This non-monotonic character of the relationship is most pronounced when there is density dependence in the sink and the difference in the intrinsic rate of increase between the habitats is large. These qualitative results apply irrespective of whether or not the local population in the marginal habitat could persist without immigration (Kawecki, 2000).

The results of this paper suggest the existence of evolutionary forces acting in the population dynamics of *I. dumosa*, such as gene flow and stabilizing selection maintaining the homogeneity in the populations from the core, in terms of fitness. The migration, colonization and divergent selection acting in the marginal population from the region 6 tend to fix the adaptation of this population to his local environment, resulting in patterns of local adaptation.

ACKNOWLEDGMENTS

I grateful acknowledge to the Drs. Massimiliano Dematteis, María Silvia Ferrucci and Viviana Solis Neffa for valuable contributions on the manuscript.

BIBLIOGRAPHY

BRIGGS, D. & S. M. WALTERS. 1997. *Plant variation and evolution*. Cambridge University Press, Cambridge.

CLAUSEN, J., D. KECK & W. M. HIESEY. 1940. *Experimental Studies of the Nature of Species. I. Effect on Varied Environments on Western North American plants*. Carnegie Ins.Wash. Publ,Washington, DC.

COMSTOCK, R. E. & R. H. MOLL. 1963. Genotype-environment interactions. *In*: Hanson, W.D. & H.F. Robinson (eds.), *Statistical Genetics in Plant Breeding*, NAS-NRC Publ. 982, Washington, D.C, pp. 164-194.

DEMPSTER, E. R. 1955. Maintenance of genetic heterogeneity. *Cold Spring Harbor Symp. Quant. Biol*. 20: 25-32.

DIAS, P. C. 1996. Sources and sinks in population biology. *Trends Ecol. Evol*. 11: 326-330.

DOBZHANSKI, T. 1955. *Evolution, Genetics, & Man*. Wiley & Sons, New York.

FELSENSTEIN, J. 1976. The theoretical population genetics of variable selection and migration. *Ann. Rev. Genet*. 10**:** 253-280.

FRANKHAM, R., J. D. BALLOU & D. A. BRISCOE. 2002. *Introduction to conservation genetics*. Cambridge University Press, Cambridge.

GALLOWAY, L. F. & C. B. FENSTER. 2000. Population differentiation in an annual legume: local adaptation. *Evolution* 54: 1173-1181.

GAUTHIER, P., R. LUMARET & A. BE´DE´CARRATS. 1998. Ecotype differentiation and coexistence of two parapatric tetraploid subspecies of cocksfoot (*Dactylis glomerata*) in the Alps. New *Phytol*. 139: 741-750.

GIBERTI, G. C. 1989. Los parientes silvestres de la yerba mate y el problema de su adulteración. *Dominguezia* 7: 1-22.

GIBERTI, G. C. 1999. Recursos fitogenéticos relacionados con el cultivo y explotación de la Yerba mate (*Ilex paraguariensis* ST. HIL., Aquifoliaceae) en el cono sur de América. *Act. Hort*. 500: 137-144.

GILBERT, G. S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Ann. Rev. Phytopathol*. 40: 13-43.

GUPTA, A. P & R. C. LEWONTIN. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. *Evolution* 36: 934-948.

HEDRICK, P. W., M. E. GINEVAN & E. P. EWING. 1976. Genetic polymorphism in heterogeneous environments. *Ann. Rev. Ecol. Syst*. **7:** 1-32.

JAKOBSSON, A. & P. DINNETZ. 2005. Local adaptation and the effects of isolation and population size – the semelparous perennial *Carlina vulgaris* as a study case. *Evolut. Ecol*. 19: 449-466.

JOSHI, J., B. SCHMID, M.C. CALDEIRA, P. G. DIMITRAKOPOULOS, J. GOOD, R. HARRIS, A. HECTOR, & T. J. KAWECKI. 2000. Adaptation to marginal habitats: contrasting influence of dispersal on the fate of rare alleles with small and large effects. *Proc. Roy. Soc. Lond. B* 267: 1315-1320.

KAWECKI, T. J & D. EBERT. 2004. Conceptual issues in local adaptation. *Ecol. lett*. 7: 1225-1241.

LENORMAND, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol*. 14: 183-189.

LEVENE, H. 1953. Genetic equilibrium when more than one ecological niche is available. *Amer. Nat*. 87: 331-333.

LINHART, Y. B. & M.C. GRANT. 1996. Evolutionary significance of local genetic differentiation in plants. *Ann. Rev. Ecol. Syst*. 27: 237-277.

MAYNARD SMITH, J. 1966. Sympatric speciation. *Amer. Nat*. 100: 637-650.

MCKAY, J.K. & R. G. LATTA. 2002. Adaptive population divergence: markers, QTL and traits. *Trends Ecol. Evol*. 17: 285-291.

NAGY, E. S. & K. J. RICE. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* 51: 1079-1089.

NETER, J., M. H. KUTNER, C. J. NACHTSHEIM & W. WASSERMAN. 1996. *Applied Linear Statistical Models*. WBC/McGraw-Hill, Boston.

PARKER, M. A. 1995. Plant fitness variation by different mutualist genotypes. *Ecology* 76: 1525.1535.

PRATI, D. & B. SCHMID. 2000. Genetic differentiation of life history traits within populations of the clonal plant *Ranunculus reptans*. *Oikos* 90: 442.456.

SLATKIN, M. 1973. Gene flow and selection in a cline. *Genetics* 75: 733.756.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236: 787.

SNAYDON, R. W. & T. M. DAVIES. 1982. Rapid divergence of plant populations in response to recent changes in soil conditions. *Evolution* 36: 289.297.

TACHIDA, H. & T. MUKAI. 1985. The genetic structure of natural populations of *Drosophila melanogaster*. XIX. Genotype-environment interaction in viability. *Genetics* 111: 43 -55.

TURESSON, G. 1922. The genotypical response of the plant species to the habitat. *Hereditas* 3: 211.350.

VAN TIENDEREN, P. H. & J. VAN DER TOORN. 1991. Genetic differentiation between populations of *Plantago lanceolata* I. Local adaptation in three contrasting habitats. *J. Ecol*. 79: 17-42.

WASER, N.M. & M. V. PRICE. 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae): evidence for local adaptation. *Amer. J. Bot*. 75: 1726-1732.

Recibido el 29 de junio de 2010, aceptado el 20 de octubre de 2010.