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Ecological and evolutionary aspects of population differentiation in three related *Cyclamen* species in the western Mediterranean

Abstract

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The Mediterranean flora contains many examples of disjunct species distributions. In the genus *Cyclamen* (*Primulaceae*) three species show disjunct distribution patterns in the western Mediterranean basin that suggest the importance of physical isolation events for speciation in the genus. *C. balearicum* occurs in five fragmented sites in southern France and on four of the Balearic Islands, *C. creticum* is endemic to Crete and Karpathos and *C. repandum* is more widely distributed from southern France to southern Greece including all the main Mediterranean Islands. The purpose of this paper is to illustrate how studies of the ecology and genetics of spatial population structure within and between species can provide important information on the role of physical isolation events for speciation. Continental populations of *C. balearicum* show extremely high levels of genetic differentiation which greatly exceed those among true island populations. Human fragmentation and/or glaciation may have drastically reduced population sizes on the continent causing such higher levels of differentiation. It is also possible that because Balearic island populations have a wider ecological amplitude than continental populations they show less differentiation. *C. repandum* also shows significant levels of population differentiation. The genetic distances among the three species indicate that this species, or an ancestral form, has given rise to *C. balearicum* and *C. creticum* at each of its distribution limits. Our data indicate that such speciation would have been facilitated by high levels of genetic isolation among populations. Variation in floral traits and levels of inbreeding within and among these species suggest that the evolution of selfing variants in the derivative species may have contributed to this speciation.

Introduction

A feature of the Mediterranean flora that has received a great deal of attention from taxonomists, ecologists and botanists is the large number of endemic species that occur in various regions of the Mediterranean basin. The current distribution pattern of many plant groups in the Mediterranean flora is one of closely related species (and/or sub-species) in geographical proximity but isolated by physical barriers. Such barriers include mountain ranges, stretches of sea and geological discontinuities and it is clear that the geological complexity of the Mediterranean basin combined with historical variations in sea level are major causes underlying the patterns of distribution of endemic plants in the

Mediterranean basin. As several contributions to this conference indicate, this is particularly apparent when one examines the flora of Mediterranean islands or zones of extreme soil composition. However, despite the fact that the Mediterranean flora provides fascinating opportunities to examine how isolation events may contribute to species diversification, empirical studies of population differentiation in relation to physical isolation and its relevance to speciation and the creation of endemic species in the Mediterranean remain few and far between (Thompson 1999). And yet, population differentiation is a necessary feature of the evolution of endemic species.

Another reason why it has become increasingly important to study patterns of population differentiation in Mediterranean plants is that in the Mediterranean basin many species now occur in habitats that are fragmented patches in a landscape fashioned by man. These terrestrial islands represent what man has left of once more continuous natural vegetation cover and are thus of immense conservation value. Such populations are often small in size and completely isolated from one another. The consequences of this fragmentation for the continued viability of Mediterranean endemics remains unknown, but will, we suggest, become a topic of increasing concern.

In our work we have begun studies on the ecology and genetics of natural populations of three closely related western Mediterranean *Cyclamen* species. Our objectives are to characterize the ecology and dynamics of continental and island populations, to quantify the effects of habitat isolation on levels of genetic differentiation and to examine the potential importance of such isolation in combination with changes in reproductive biology for patterns of species diversification.

The three spring-flowering Cyclamens

The genus *Cyclamen* (*Primulaceae*) contains 20 species of which 16 grow under a Mediterranean climate. The three species we have studied all flower in the spring (March-April) and show an interesting pattern of disjunct distributions (Fig. 1). *Cyclamen balearicum* Willk. (2n=20) is endemic to the Balearic Islands (Mallorca, Menorca, Ibiza and Cabrera) and southern France, *C. creticum* Hildebr. (2n=20 or 22) is endemic to the closely adjacent islands of Crete and Karpathos and *C. repandum* Sm. & Sibth. (2n=20), is widely distributed from southern France to southern Greece (Grey-Wilson 1997) with an isolated group of populations in Algeria (Debussche & Quézel 1997).

All three *Cyclamen* species studied are long-lived, perennial herbs with a tuberous rootstock. None of the species show evidence of vegetative reproduction. The long-petiolate leaves arise directly from the tip of the floral trunk which emerges from the upper side of the tuber. The hermaphroditic flowers are solitary and pendant at the tip of an erect leafless pedicel emerging from the floral trunk. They do not produce nectar. The corolla is divided into five reflexed lobes and the five sepals are fused at the base. Anthers are positioned inside the corolla and attached to the base of the corolla by a very short filament. Anther dehiscence is introrse and pollen is mainly liberated through an apical pore. The superior ovary contains numerous ovules. After fertilization the corolla falls and the erect pedicel coils from the apex downwards bringing the capsule to the substrate surface where the brownish seeds are liberated and dispersed by ants (e.g. Affre & al. 1995).

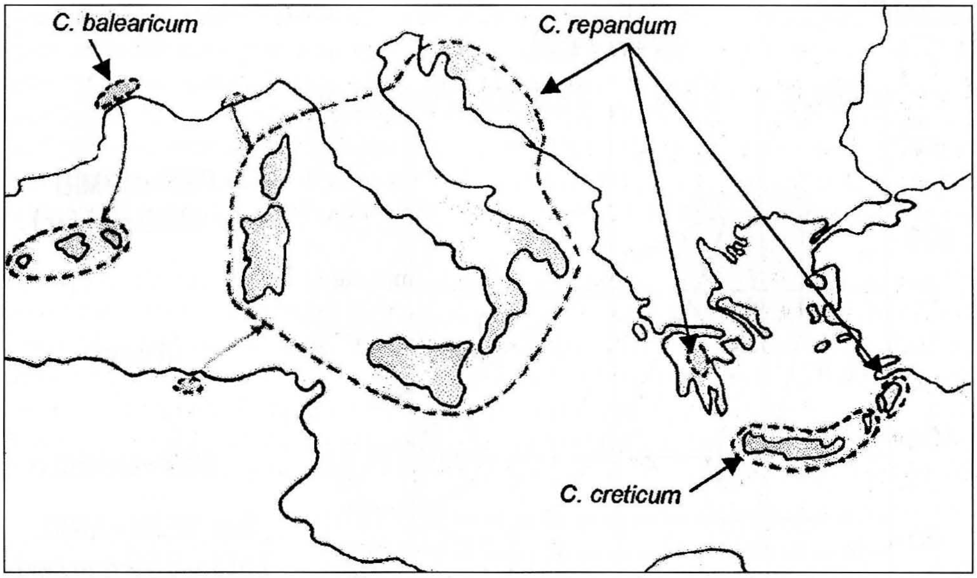

Fig. 1 The distribution of the three study species.

The ecology of *Cyclamen balearicum*

An initial stage in the examination of evolutionary processes in relation to spatial isolation demands an answer to the question: where does the species occur? It is not always a simple matter to accurately describe the full extent of a species' distribution. For example, long-established and reliable publications indicating the true extent of the distribution of *C. balearicum* in southern France have not been referred to in recent floras and descriptions of the genus *Cyclamen* sometimes because of taxonomic errors. As a result, the two sites in the extreme south-east of the distribution (Minervois and Corbières) were overlooked and the true limits to the distribution of the species were not accurately defined. Recent observations (Debussche & al. 1995) have confirmed the persistence of *C. balearicum* in each of the five zones (and in some cases documented a much larger number of local populations than previously documented) and thus we now have a true picture of the distribution of the species on the continent.

Despite its restriction to the Balearic islands and to small terrestrial islands of suitable habitat on the continent, *C. balearicum* occupies a wide range of climatic conditions, due to the differences in winter temperature between France and the Balearic Islands. As a result, *C. balearicum* can be found growing not only alongside *Primula veris, Sanicula europaea* and *Tilia platyphyllos* in southern France but also with *Chamaerops humilis* and *Nerium oleander* on the Balearic islands. *C. balearicum* is restricted to basic soils on limestone, except for a few populations on Menorca which grow on slightly acidic soils on schistous rocks. Populations on the Balearic islands have a greater ecological amplitude than those in southern France (Debussche & al. 1995, 1997). We have thus begun to test the hypothesis that island populations have a broader ecological amplitude as a result of niche enlargement in insular environments.

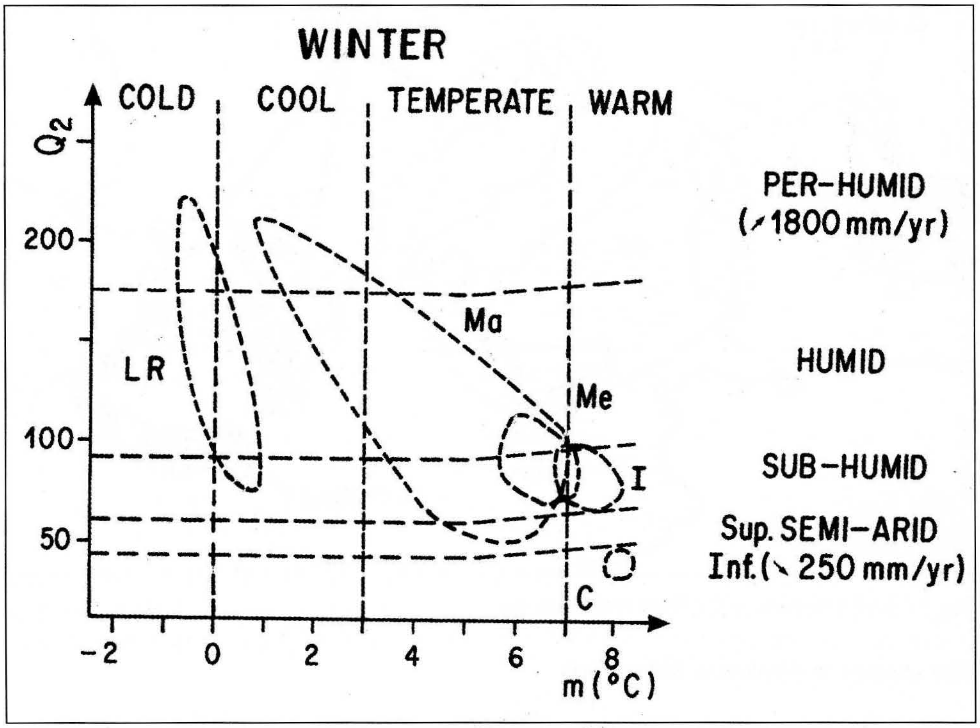

Fig. 2 The climatic amplitude of *C. balearicum* expressed on an Emberger bioclimatic diagram (following Debussche et al. 1996; 1997). LR: Languedoc-Roussillon in southern France, MA: Majorca, Me: Menorca, I: Ibiza, C: Cabrera.

Population differentiation in *C. balearicum* and related species

What is apparent from our studies and observations on the distribution of *C. balearicum* is that this species has a markedly fragmented pattern of distribution. Particularly interesting here is the fact that *C. balearicum* occurs both (1) on several true islands in the Mediterranean sea which have been separated by ancient changes in sea level (Majorca, Menorca, Cabrera and Ibiza, and see Fig. 1) and (2) in several isolated and discrete terrestrial islands of suitable habitat on the continent in southern France (Debussche & al. 1995). We have thus been able to compare the effects of habitat fragmentation and spatial isolation in continental populations with island isolation on the Balearic islands.

We quantified population differentiation by calculating Wright's fixation indices based on isozyme electrophoresis of several polymorphic loci (see Affre & al. 1997 for details). The major result of this study (see Fig. 3) was that the amount of differentiation among true islands was significantly ($P < 0.05$) less than that among the terrestrial islands and that differentiation among populations from a single true island (Majorca) was significantly less than that observed among populations on one of the habitat islands (Cévennes). This was *a priori* against our predictions; given that true islands have been isolated for several millions of years we expected there to be more differentiation among the Balearic islands than among

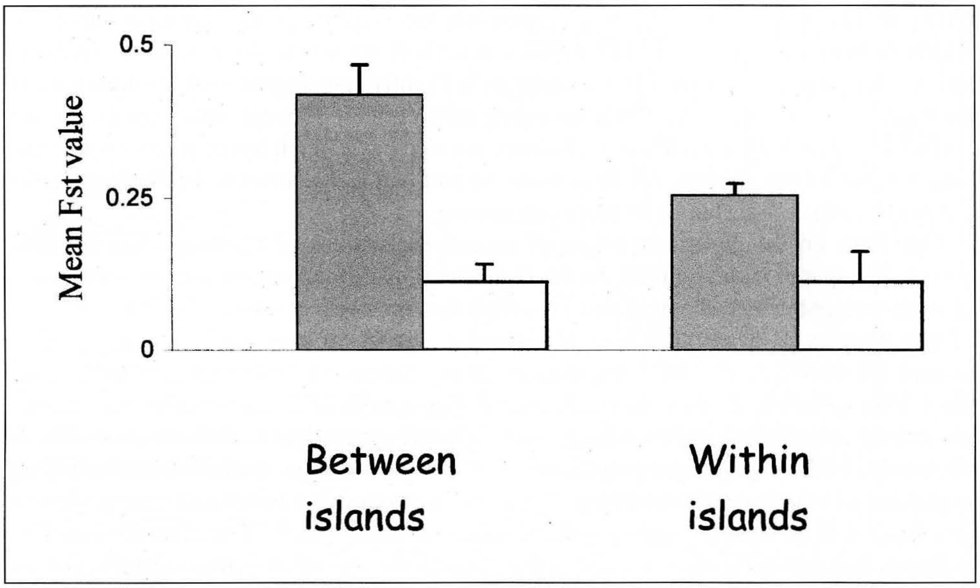

Fig. 3 Estimates of differentiation (Fst) among C. balearicum populations from the Balearic islands (open bars) and terrestrial habitat islands (closed bars) at two geographical scales, among islands and among populations on a single island.

terrestrial habitat islands where the probable cause(s) of isolation, i.e. glaciation and/or fragmentation by man, means that they have been isolated for a shorter period of time (probably between 200-500 years). Our results indicate that the two types of isolation have not had the same effect. Two possible causes can be suggested for this differential pattern of population differentiation. First, the effects of isolation on the continent may have been more severe, reducing population sizes to extremely small relicts in fragments of suitable habitat and thus increasing the differentiation among populations due to the loss of diversity in each population. Second, the ecological conditions of populations in southern France are different to those on the Balearic islands. The latter have a more benign winter climate and it is possible that the clearing of forest has had a greater effect on populations in France where freezing in winter is more severe (and exacerbated by removal of the forest vegetation).

Studies of *C. repandum* on Corsica and *C. creticum* on Crete (Affre & Thompson 1997a, b) have shown that these species also have significant levels of population differentiation among island populations (F_{st} = 0.42 and 0.17 respectively). These studies indicate that the basis of species divergence due to physical isolation exists, i.e. significant population differentiation and thus low levels of gene flow. We have thus now turned our attention to features of the natural biology and ecology of these *Cyclamen* species that may have accompanied and perhaps contributed to species divergence.

Mating system variation and the evolution of the endemic *C. balearicum*

A major difference between the three *Cyclamen* species concerns their floral phenotype

(Affre & Thompson 1998). Whilst *C. repandum* has large magenta - crimson flowers in which stigmas are longer than the corolla and anthers are within the corolla. *C. creticum* has similar sized flowers which are white with slightly less stigma anther separation. In contrast, *C. balearicum* meanwhile has much smaller white flowers with stigmas positioned almost level with the anthers. *C. balearicum* also has a much lower pollen-ovule ratio than the two former species. All these traits suggest that *C. balearicum* is a selfing species whereas *C. repandum* should be more outcrossing.

Our work on the genetic structure of natural populations of *Cyclamen* has not only shown that spatial isolation may permit high levels of differentiation among populations of each species but has also provided important data on levels of inbreeding in populations of these species. *C. balearicum* is highly inbred, F_{is} values for individual populations being around 0.9 (Affre & al. 1997). Populations in the Balearic Islands and southern France show little variation, they are all highly inbred. Populations of *C. creticum* have F_{is} values around 0.6 and are thus highly inbred, but to a lesser extent than *C. balearicum* (Affre & Thompson 1997b). In contrast populations of *C. repandum* show marked variation among populations in the level of inbreeding, F_{is} values varied from 0.1 to 0.8 and were positively correlated with altitude for eight populations on Corsica (Affre & Thompson 1997a). This indicates that inbreeding has evolved at the altitudinal limits of the species distribution on the island of Corsica.

These data concord with the ecology, floral biology and pollination ecology of the different species. *C. balearicum* occupies a type of habitat where pollinators are very rare, indeed many hours of observations in the field have shown that insect visits to this species are extremely rare. In the absence of pollinators this species can self-pollinate due to the proximity of the stigma to the anthers and the orientation of the flower (downwards) which allows pollen to fall on the stigma (Affre & al. 1995). The genetic data suggest that this is probably the main mode of reproduction in this species and we have found that this species is self compatible and shows little negative effect of inbreeding on offspring performance (Affre & Thompson 1999). In contrast, due to the spatial separation of stigmas and anthers, *C. repandum* sets very little seed in the absence of pollinators (Affre & Thompson 1999). However, like *C. balearicum* the species is self-compatible and seed produced from hand-selfing do produce viable adult plants (Affre & Thompson 1999). *C. repandum* is visited primarily by bumble bees (J. D. Thompson & L. Affre unpublished data) which, whilst hanging from the corolla, extract pollen from the anthers by vibrating the flower with their wing muscles - a mode of pollination known as buzz pollination.

Finally, a comparison of genetic distances among the three species based on isozyme (supposedly neutral) markers has shown that *C. creticum* and *C. balearicum* are less similar to each other than they both are to *C. repandum*. This is highly suggestive of an independent evolution of the two former species from a common ancestor similar to contemporary *C. repandum* at each of its distribution limits (see Fig. 1). Current work on the molecular phylogeny of the genus supports this claim (L. Geilly & J. D. Thompson unpublished data). In contrast, based on morphological characters, *C. creticum* and *C. balearicum* are more similar to each other than they are to *C. repandum*. This indicates that during or since the speciation of the two former species, the evolution of floral characters has followed a similar trajectory. The evolution of selfing variants may thus have been of selective advantage and facilitated the establishment of populations of these two species. What is more, *C. repandum* populations occasionally contain plants with smaller white flowers similar to the two derivative species intermingled with plants of the normal *C. repandum* phenotye (J. D. Thompson, L. Affre & M. Debussche unpublished data). Hence the potential for the evolution of a new species with this type of floral morphology via population isolation and loss of pollinators exists. *C. repandum* thus appears to have evolved towards high levels of inbreeding via a major change in floral phenotype and, as we pointed out above, via more gradual increases in inbreeding at the altitudinal limits to its distribution on Corsica.

Conclusion

The juxtaposition of data on mating system variation, quantitative measures of floral traits, pollinator observations, population differentiation and genetic distances between species indicate that mating system changes may be closely associated with speciation in these species of *Cyclamen.* The current disjunct distribution of the three species clearly illustrates the importance of physical isolation in this context. The three species can when inter-crossed produce viable seed (J. D. Thompson unpublished data) hence the importance of such isolation for species integrity in this group. Another important feature of our results is the observation that *C. repandum* occurs primarily on acidic substrate but with some rare populations on limestone (M. Debussche unpublished data) whilst *C. balearicum* occurs almost exclusively on limestone (Debussche & al. 1995, 1997). Hence the potential for ecological adaptation in the divergence of the two species.

Finally, *Cyclamen* species represent an important component of the heritage of Mediterranean plants, emblematic to many botanists. They are also of important commercial interest and have been for over a hundred years. Yet we have only recently begun to understand the ecology and biology of natural populations and to document the true extent of diversity in the genus. There is clearly much scope for future work on disjunct distribution patterns that can be observed in *Cyclamen* and in many Mediterranean plant groups due to the insight such patterns provide into the evolution of the Mediterreanean flora.

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