

Origin and Maintenance of *Rhododendron x sochadzeae*, a Fertile F1 Hybrid which Occupies an Ecotone between *R. ponticum* and *R. caucasicum* in Turkey

Richard I. MILNE

Division of Environmental and Evolutionary Biology, School of Biology, University of St Andrews, St Andrews, Fife, KY169 TH, Scotland, UK

Salih TERZİOĞLU

Karadeniz Technical University, Faculty of Forestry, Department of Forest Botany, 61080 Trabzon - TURKEY

Richard J. ABBOTT

Division of Environmental and Evolutionary Biology, School of Biology, University of St Andrews, St Andrews, Fife, KY169 TH, Scotland, UK

Received: 10.09.2002

Accepted: 29.01.2003

Abstract: Isolating barriers between sympatric interfertile species are maintained by processes that occur within their hybrid zones. Although the effects of intrinsic selection on hybrid fitness are well known, less is understood about extrinsic fitness variation. At Tiryal Dağı, NE Turkey, *Rhododendron x sochadzeae* Charadze & Davlianidze (*R. ponticum* L. x *caucasicum* Pall.) forms large populations in which neither segregation nor backcrossing occur, in habitats intermediate between those of its parents. Using single-copy species-specific RAPD and ISSR markers, it was determined that all *R. x sochadzeae* plants are F1s, and that there are many separate genets present. Through hand pollination experiments, it was determined that *R. x sochadzeae* plants can produce viable F2 seeds or backcrosses in either direction. Therefore, all non-F1 hybrid derivatives appear to be eliminated at Tiryal Dağı due to post-germination selection. However, adult backcrosses can and do recruit in large numbers in different habitat conditions at another site. From this, the selection that favours F1s over BCs at Tiryal Dağı must be habitat-mediated. We have concluded that strong habitat-mediated selection is most likely maintaining species barriers at Tiryal Dağı by eliminating hybrid generations subsequent to F1. Similar forces might occur in other hybrid zones, but may be countered by additional effects that increase fitness in post-F1s, or restrict the formation and/or fitness of F1s. We suggest that the superior fitness of F1s might be due to gene complexes that confer tolerance to specific habitat conditions in each parent that break up after the F1 generation. A simple mathematical model is provided, that describes how an F1 might thereby outcompete subsequent generations at all points along a habitat gradient.

Key Words: Species barriers, reproductive isolation, hybrid zones, extrinsic fitness, F1 advantage, hybrid breakdown, gene complexes, *Rhododendron*

Definitions of species used by evolutionary biologists range from the rigid interpretation of the biological species concept, i.e. that no 2 populations should be considered as separate species if they are capable of forming fertile hybrids and so interbreeding, to those that allow interbreeding provided there are clear and consistent morphological differences (Mallett, 1995). Plant species are often capable of remaining separate in the wild even where fertile hybrids are produced in large numbers (Rieseberg & Wendel, 1993; Arnold, 1997; Rieseberg & Carney, 1998). Therefore, to properly understand species requires an accurate understanding of the processes that keep them separate in the wild, which in turn requires the study of hybrid zones.

Most documented plant hybrid populations fall into 2 types, although there is a continuum of variation between these. At one extreme are those hybrid populations that consist only of a few entirely sterile F1s, in which case hybridisation can proceed no further. At the other extreme are populations that comprise large numbers of hybrid individuals, many or most of which are of advanced generations. Even the very rare production of an almost sterile F1 can lead to the formation of such a large hybrid population (Arnold, 1997, 2000). The parent species may have a variety of pre- and post-mating mechanisms that minimise hybrid formation. However, once an F1 has been formed, often none of these mechanisms will prevent the F1 from receiving, and being

fertilised by, pollen from one or both parents. Therefore, provided that the F1 is at least partly fertile, a second hybrid generation will often form (Arnold & Hodges, 1995; Arnold, 1997, 2000). Moreover, in cases where the fertility and/or viability of F1s is low, these traits often increase in each successive hybrid generation (Rieseberg, 1997). Therefore if the F1 sets viable seed at all, this will provide a second generation of hybrids that might be more fertile than the first. These will breed among themselves and with parent taxa, providing a third hybrid generation which might comprise more and fitter individuals again than the second. In this way, a large hybrid population can arise over relatively few generations. In other cases, the hybrid population might consist of an F1 and a limited number of backcrosses.

Among such large hybrid populations, the range of morphological variation often exceeds the combined variation range of the parent species. This is due to segregation (the break-up and mixing of parental genomes after the F1 generation) and epistasis (the combined effects of heterospecific genes) (Rieseberg & Ellstrand, 1993; Rieseberg et al., 1999). These phenomena can also have profound effects (both positive and negative) on hybrid fitness. A large hybrid population is therefore usually a mix of hybrid derivatives, with variable fitness and fertility between individuals, and a range of genetic compositions from individuals similar to one parent, through intermediate individuals (which are often rare) to those approaching the second parent species.

Hybrid populations of this kind can be responsible for the transfer of genes between species. This normally occurs through repeated backcrossing towards one parent, so that genes from the other parent combine into the first's genetic background (introgression; see Rieseberg & Wendel, 1993, for a review). Patterns of mating between hybrids and parent individuals, and of fitness and survival among hybrids, will determine the extent to which this occurs. It is possible that beneficial genes may move across species barriers in this fashion, if they confer a selective advantage irrespective of genetic background.

A key factor governing gene flow across hybrid zones is the fitness of hybrids relative to the parent taxa, and of hybrid individuals relative to each other. Plant fitness has 2 components: intrinsic (habitat-independent) and extrinsic (habitat-dependent). Intrinsic hybrid fitness can

be reduced in hybrid derivatives due to the break-up of co-adapted gene complexes (Dobzansky, 1970; Li et al., 1997) and incompatibilities between the parental genomes. This alone can restrict both gene flow and the extent of the hybrid population (Barton & Hewitt, 1985). However, strong associations between hybrid genotypes and specific habitat types have indicated that in many cases the distribution of hybrids is at least partly controlled by extrinsic fitness (Arnold, 1997, 2000). In particular, hybrids which are genetically similar to one parent tend to be found in habitat conditions approaching those of the same parent species (Anderson, 1949; Arnold & Hodges, 1995). Both fitness components are important, but intrinsic fitness is much better understood. Intrinsic fitness effects tend to be more prevalent in hybrids between species that are only partly interfertile and compatible. Therefore, the mechanisms that maintain species barriers between highly interfertile plant species are relatively poorly understood.

In the mountains of NE Turkey, particularly the seaward slopes that receive most rainfall, grow 4 closely related *Rhododendron* L. species, all of which are interfertile with one another (Chamberlain, 1982; Milne et al., 1999). A fifth taxon, which makes up a substantial component of the vegetation in this area, is *Rhododendron x sochadzeae* Charadze & Davlianidze. The parents of this hybrid are *R. ponticum* (L.) Schreb. ex DC. and *R. caucasicum* Pall., which are respectively low (mostly <2000 m) and high (mostly >2000 m) altitude species. *R. x sochadzeae* forms extensive populations wherever their distributions overlap, on mountain slopes between 1800 and 2200 m.

There is scarcely more morphological variation among populations of *R. x sochadzeae* than among either parent species (Milne et al., 1999; Güner and Duman, 1998; Stevens, 1978). This indicates that, in contrast to the normal situation described above, segregation is not occurring. Moreover, there is no intergradation of morphological forms between *R. x sochadzeae* and its parents, which indicates that backcrossing is not occurring. Furthermore, the plants appear to set viable seed, so they are not sterile F1s, and have the same chromosome number ($2n = 26$) as the parents. The *R. x sochadzeae* population therefore has a very different population structure from other described hybrid zones.

One possible explanation for this lack of variation within *R. x sochadzeae* is that reproduction might be

clonal. However, subtle morphological differences between bushes, in characters such as bark patterning, corolla shade and leaf colour, indicate that they might be different genets. This has recently been confirmed using molecular markers (Milne et al., in press). Therefore clonal reproduction, although it does occur, cannot account for the large populations of morphologically similar plants that exist.

Three further possible explanations remain. First, the population might comprise one or few selfing lineages. Second, the population might comprise a large number of F1 individuals. The final possibility is that *R. x sochadzeae* is actually a hybrid species, which breeds among itself and is adapted to the narrow altitude range between those of its parent species. Other examples of homoploid (i.e. without chromosome doubling) hybrid species developing from hybrid populations are known (Rieseberg, 1997) including one that has adapted to an altitude intermediate between those of its parents (Brochmann et al., 2000).

These 3 hypotheses could all be distinguished using molecular markers that are specific to the parent species, present in single copies, and subject to simple Mendelian inheritance (Milne et al., in press). Ten such markers have been developed, with 5 specific to each parent species. A selfing lineage would be almost completely homozygous, which would cause about 50% expression of parent-species specific markers. Conversely, an F1 would always be heterozygous for every parent-specific marker, so 100% of these would be expressed. A stabilised hybrid species would have all neutral parent-specific markers in HW-equilibrium, i.e. ~50% homozygosity, causing 75% of parental markers to be expressed. Surprisingly, all *R. x sochadzeae* individuals examined have been shown to be heterozygous for all markers, indicating unequivocally that they were all F1s (Milne et al., in press).

The ability of *R. x sochadzeae* to interbreed with its parent species has also been tested. This was done by hand-pollinating flowers of 12 *R. x sochadzeae* individuals with each of *R. caucasicum*, *R. ponticum*, and another *R. x sochadzeae* plant. Since *Rhododendrons* are protandrous, flowers to be pollinated had to be emasculated and bagged when not quite open, and pollen was applied directly to the stigma 4 days later, when it was ripe. Treated flowers of *R. x sochadzeae* mother plants were marked with coloured tape on the pedicel or stem to indicate which father taxon was used.

Apart from pods that suffered physical damage or predation, virtually every treated flower produced a seed pod, and these were collected during a return visit at the beginning of October. All 3 treatments produced seed in copious amounts (Milne et al., in press). Some pods that had formed naturally were also collected, and these also contained large quantities of viable seed. Therefore, viable seed of the second hybrid generation can be and is produced in these populations.

Overall, therefore, the results of studies recently undertaken have indicated that an almost unprecedented situation exists in the *R. x sochadzeae* population. The population is comprised of a large number of F1s, each independently produced, and each capable of forming viable seed of the second hybrid generation in substantial quantities. The only conclusion that may be drawn from these observations is that the second hybrid generation is eliminated from the population by very strong selection. Studies on *R. ponticum* in the UK have indicated that *Rhododendron* seedlings are far more susceptible to both competition and abiotic stresses in their first few years of life than later on (Cross, 1975; Simons, 1988). From this, most selection probably occurs at the early seedling stage, which has also been asserted for other populations of hybridising shrubs (Wang et al., 1997).

As yet, there is no direct evidence available regarding the fitness and performance of the different genotype classes. From the association of genotypes with habitats, however, strong inferences can be made about the factors that maintain the *R. x sochadzeae* population. The first is that F1s are superior to both the parent species within the hybrid habitat. On valley side slopes at ~2000 m, large populations of *R. x sochadzeae* form to the exclusion of all other *Rhododendron* species. Equally, the populations of *R. caucasicum* in wet valley bottoms contain no plants of *R. x sochadzeae*; and among *R. ponticum* on ridge tops, *R. x sochadzeae* is either absent or occurs at extremely low frequency, possibly on locally moist microsites. From this, the superiority of F1s over parents is almost certainly habitat-mediated.

Such F1-dominated hybrid populations appear to be the norm for *R. x sochadzeae*, because low levels of morphological variation between individuals of this taxon have been observed throughout NE Turkey (Milne et al., 1999; Güner & Duman, 1998; Stevens, 1978). However, an atypical (for this combination) hybrid zone between *R. ponticum* and *R. caucasicum* was observed at

Çamlıhemşin, Turkey (Milne et al., 1999). This hybrid population existed at c. 1600 m (i.e. below the usual range of either *R. caucasicum* or *R. x sochadzeae*). The site was a stream running through a steep-sided, steeply sloping, and north-facing valley. The stream-side sites were damp, mossy and covered through spring by late-lying snow; in such sites occasional plants of *R. caucasicum* were found, together with many hybrid plants. These locally cold conditions might have assisted *R. caucasicum* and prevented *R. ponticum* from germinating. On either side of the stream these wet habitats were abruptly replaced by steep dry rocky slopes dominated by *R. ponticum*, including occasional plants showing introgression from *R. caucasicum*. Among the streamside hybrids, a far greater range of morphological variation occurred than at Tiryal Dağı, despite the much smaller number of plants. Hence, segregation and backcrossing appeared to be occurring freely at this site, and F1s were rare (Milne et al., 1999). The population at Çamlıhemşin provides clear evidence that backcrosses can form adult plants in the correct conditions. From this, the fitness disadvantage that removes backcrosses from the Tiryal Dağı population might be habitat-mediated, rather than intrinsic. Therefore, F1s at Tiryal Dağı appear to have extrinsic superiority over all other genotype classes.

There is one other documented instance of a hybrid population that is dominated by fertile F1s. *Encelia laciniata* Vasey & N.E.Rose. is the hybrid between *E. ventorum* Brandegee, which occurs on coastal sand, and *E. palmeri* Vasey & N.E.Rose., an inland species (Kyhos et al., 1981). It occurs commonly where the parent species are parapatric, and usually forms multi-generation hybrid swarms. However, on one site - the leeward slope of a fixed sand dune - a population dominated by fertile F1s was observed (Kyhos et al., 1981). As with *R. x sochadzeae*, viable seed was produced by these F1s, and the absence of BCs and F2s could "only be accounted for by absolute post-dispersal selective forces preventing their survival" (Kyhos et al., 1981). Furthermore, as with *R. x sochadzeae*, from the morphology of nearby parent populations, little or no introgression was occurring in the vicinity of the F1 hybrid population. However, in contrast to *R. x sochadzeae*, only one F1-dominated population of *E. laciniata* has been observed, and multi-generation hybrid swarms are more common for this hybrid. This might reflect differences in the type of habitat that is commonly available in the 2 cases.

Since they differ in structure from either hybrid populations that comprise only sterile F1s, or those that contain hybrid derivatives of many generations, the hybrid populations of the types found in *R. x sochadzeae* and *E. laciniata* have been termed the F1-dominated hybrid zone (F1DZ). Two further putative examples of F1DZs may occur in Austria; these are *Rhododendron x intermedium* Hegetschw. and *Salvia x sylvestris* L. (Kerner, 1895). F1DZs are characterised by large numbers of fertile F1s and the absence of later generation hybrid derivatives. They tend to occur on undisturbed habitats that are transitional between those of the parent species.

Habitat-mediated superiority of a range of hybrid classes over the parent species has been indicated in a few cases (Wang et al., 1997; Arnold & Bennett, 1993; Johnson et al., 2001). Furthermore, F1 superiority across all habitats has been noted (Emms & Arnold, 1997; Burke et al., 1998), which might be due to combined extrinsic and intrinsic fitness advantages. Conversely, F1 superiority over BCs is a phenomenon thus far only recorded with certainty with regard to intrinsic fitness. It can occur through hybrid breakdown following segregation, which in turn is attributed to the break-up of co-adapted gene complexes (Dobzansky, 1970; Li et al., 1997; Rieseberg & Carney, 1998). There has not yet been a convincing demonstration of lowered extrinsic fitness in BCs relative to F1s. However, this could occur hypothetically if co-adapted gene complexes within the parent species conferred extrinsic, rather than intrinsic, fitness. Such complexes would be combined in the F1, giving tolerance of both species' habitat conditions. They would, however, be broken up during segregation in the second hybrid generation, reducing tolerance of these conditions in post-F1 generations.

In the case of *R. x sochadzeae*, *R. ponticum* might contain a complex conferring tolerance to drought or other low-altitude conditions; *R. caucasicum* might contain a complex conferring tolerance to cold, or other high altitude conditions. If so, the F1 would contain a complete set of the gene complex conferring tolerance to each condition. This would explain the F1s (*R. x sochadzeae*) outcompeting both parent taxa, consistently, on semi-moist slopes at intermediate altitudes, where tolerance to both conditions might be required for high fitness. Similarly, backcrosses might be outcompeted in these habitats because they possess a complete set of one but not both gene complexes.

At the edge of the hybrid zone, where conditions approach those of one parent species, conditions usually favour backcrosses, whose genome is more similar to that of parent species (Anderson, 1949; Arnold & Hodges, 1995). However, in the *R. x sochadzeae* population, F1s dominate the entire range of habitats, from that dominated by one parent species, to that dominated by the other. To demonstrate how co-adapted gene complexes might account for the dominance of F1s not only at the centre of the hybrid zone, but also at its edges, a simple mathematical model was devised. The assumptions of the model are as follows:

- (1) The extrinsic fitness score of any individual is determined by a component of cold tolerance (CT) plus a component of drought tolerance (DT). All other fitness traits are assumed to be equal between all individuals and genotype classes.
- (2) The environment applies a component of cold stress (CS) and a component of drought stress (DS) such that $CS + DS = 1$. The hybrid zone habitat is an ecotone, and grades smoothly from $CS=1$ at one extreme, to $DS=1$ at the other.
- (3) The overall extrinsic fitness of an individual is determined by $(CT*CS) + (DT*DS)$. Hence the fitness advantage conferred by cold- and drought-tolerance traits is proportional to the degree of cold and drought stress in the environment, respectively.
- (4) *R. ponticum* contains a complex of 8 genes conferring drought tolerance. Call the number of genes present (in homo- or hetero-zygous state) NP.
- (5) *R. caucasicum* contains a complex of 8 genes conferring cold tolerance. Call the number of genes present (in homo- or hetero-zygous state) NK.
- (6) Each parent species is homozygous for all genes in its complex, and genes from each complex display simple Mendelian inheritance and dominant expression. There is no linkage between these genes.
- (7) Genes from each complex enhance the effect of every other, such that cold and drought tolerance effects are proportional to the square of the number of genes from each complex expressed.

- (8) The effectiveness of each gene complex is also affected by the proportion of heterospecific germplasm present, such that effectiveness grades smoothly from 100%, in a background of only homospecific germplasm, to 50%, in a hypothetical background of entirely heterospecific germplasm. Call RP the proportion of *R. ponticum* germplasm.

Therefore, cold-tolerance effect $CT = NP^2 * (2 - RP)/2$
and, drought-tolerance effect $DT = NK^2 * (1 + RP)/2$

Therefore the overall extrinsic fitness score at any point on the habitat gradient is given by

$$[(NP^2*(2 - RP)/2)*CS] + [(NK^2*(1 + RP)/2)*DS]$$

From this, the fitness of 6 genotype classes across a habitat gradient between conditions of high drought stress and no cold stress, to the opposite, were calculated (Figure 1). The parent species possess all 8 genes of their own gene complex, but none of the other. An F1 possesses a single copy of all genes from both complexes, but because of the germplasm background effect is less fit than one or other parent where either the cold (CS) or drought stress effect (DS) is > 0.75 (Figure 1). The F2 is assumed to express 6 out of 8 genes from each complex, because it will only be heterozygous for 50% of parent-species-specific genes. It therefore has inferior fitness to the F1 across all habitats in the model (Figure 1). A backcross will possess all genes from one parent in at least one copy, but on average only 4 from the other. This means that its fitness will change across the hybrid zone, but less steeply than that of a parent species. Crucially, there is no point across the zone at which a backcross is fitter than all of the F1 and the 2 parent species (Figure 1). The model therefore indicates how backcrosses might fail due to extrinsic selection. Although the model is almost certainly an oversimplification of the real situation it does at least provide a working hypothesis for what might be happening.

Clearly, further research is required to test the hypothesis that gene complexes determine habitat-mediated fitness in *R. x sochadzeae*. Habitat-mediated

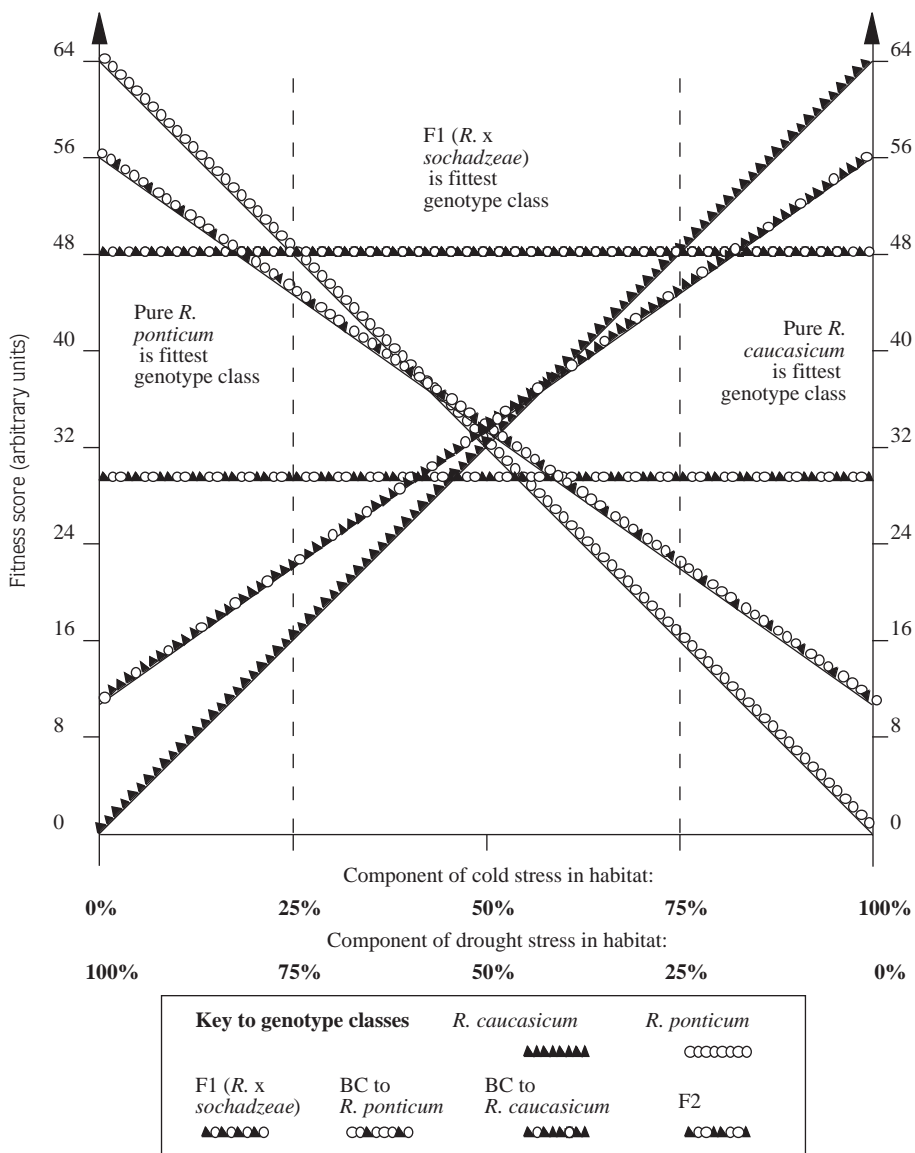


Figure 1. Fitness of 6 genotype classes across a habitat grading from one that applies strong cold-stress to one that applies strong drought-stress, according to a simple mathematical model.

superiority of F1s, whether determined by gene complexes or otherwise, could only cause F1DZ formation if 3 other conditions were also met. First, F1 seed must be produced in sufficient numbers to populate the hybrid zone. *Rhododendrons* are highly interfertile and produce tens of millions of seeds per individual's lifetime (Cross, 1975), which likely means that ample F1s are produced. However, in other cases F1 formation is extremely rare (Arnold, 1993, 1997, 2000), and probably therefore too rare for F1DZ formation to be possible.

Second, an F1DZ will not form if BCs have an intrinsic advantage over F1s that cancels out any extrinsic disadvantage. If intrinsic fitness varies greatly among BCs, which might often be the case (Rieseberg & Carney, 1998), then a few BCs will have higher fitness than F1s, and a population comprising only F1s could not, therefore, form.

Third, the extrinsic superiority of F1s might only apply in certain habitats. The model described above demonstrates that F1s might be superior to BCs over a

complete range of habitats grading from conditions to which one parent is adapted, to those that suit the other. However, it does not account for conditions to which neither parent is adapted (novel conditions). The tolerance of novel conditions can be created by epistasis after segregation (Rieseberg et al., 1999), and is thus a frequent characteristic of individual post-F1 hybrid derivatives. Therefore, the presence of novel conditions in the hybrid habitat might favour BCs over F1s. Hence novel conditions might apply at Çamlıhemşin, where BCs were abundant, but not at Tiryal Dağı, where they were absent. Therefore, F1DZ formation might require a habitat that combines the conditions of the parents' habitats, but applies no novel ones.

It is probably very rare that all 3 of these conditions are met in natural hybrid zones. In particular, a large proportion of studied hybrid zones occur on sites subject to habitat disturbance, which is a likely source of novel conditions and microhabitat variation. The parent species of *Encelia laciniata* formed multi-generation hybrid swarms in 4 sites subject to habitat disturbance, but formed an F1DZ in a single site where there was no disturbance. From this, habitat-mediated F1 fitness advantage effects might be widespread among plant hybrid populations, but rarely lead to F1DZ formation. F1DZs provide an unprecedented opportunity to study this effect, and to test the hypothesis that it is caused by gene complexes that confer habitat-tolerance. Such work might greatly enhance our understanding of how adaptation to different habitats can create species barriers between highly interfertile, parapatric plant species.

Future studies on F1DZs will need to involve tests of the fitness of various genotype classes (parents, F1, F2, BC1) in a range of habitat conditions that are novel to one or both parents. In addition, studies that involve selective breeding to restore tolerance of parental habitat conditions in hybrid derivatives, combined with QTL analysis, would be highly informative. Unfortunately, this would require identification of an F1DZ with a shorter generation time than *R. x sochadzeae*. For this reason, it

is highly desirable that further examples of F1DZs be identified. They are likely to be encountered in countries such as Turkey where large regions of vegetation exist that are relatively undisturbed.

Putative F1DZs can be easily recognised by the large numbers of hybrid individuals, the low degree of morphological variation between individuals, and especially by the absence of backcrosses towards either parent. They should be sought in undisturbed transitional habitats between those occupied by 2 parents species known to be highly interfertile. In fact, an F1DZ looks more like a separate species from its parents than it does a typical hybrid zone. The existence of an F1DZ can be unequivocally proved using molecular markers (Milne et al., in press; and see above). Without molecular markers, a strong presumption that a hybrid population is an F1DZ can be made if a morphometric analysis shows that the hybrids do not intergrade with the parent species, and if the hybrids are shown to set viable seed. The distribution of morphological characteristics across an F1DZ will normally be tri-modal, whereas in other hybrid swarms either di-modal (where most individuals approach one or other parent), or multi-modal (where no phenotype predominates) distributions of characters will be observed.

The study of *R. x sochadzeae* has demonstrated the importance of habitat-mediated selection in hybrid zones, and that genome-habitat interaction can act to prevent interspecific gene flow by selection against the second hybrid generation. Further research on F1DZ-forming plant species might provide new insights into the genetics of barriers between highly interfertile species. To this end, further examples of F1DZs need to be sought.

Acknowledgements

The authors thank H. Yiğit and E. Kahraman (Murgul Forest Enterprise, Artvin, Turkey) for accommodation during fieldwork. This work was supported by Natural Environment Research Council (NERC) grant NER/B/S/2000/00330 to R.J. Abbott and R. I. Milne.

References

- Anderson E (1949). Introgressive hybridization. New York: John Wiley and Sons.
- Arnold ML (1997). Natural hybridisation and evolution. Oxford: Oxford Univ. Press.
- Arnold ML (2000). Anderson's paradigm: Louisiana Irises and the study of evolutionary phenomena. *Mol Ecol* 9: 1687-1698.

- Arnold ML & Bennett BD (1993). Natural hybridization in Louisiana Irises: Genetic variation and Ecological Determinants. Pp. 115-139. In: Harrison RG (ed.). Hybrid zones and the evolutionary process. Oxford: Oxford Univ. press.
- Arnold ML & Hodges SA (1995). Are natural hybrids fit or unfit relative to their parents? *Trends Ecol Evol* 10: 67-71.
- Barton NH & Hewitt GM (1985). Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* 16: 113-148.
- Brochmann C, Borgen L & Stabbetorp OE (2000). Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Pl Syst Evol* 220: 77-92.
- Burke JM, Carney SC & Arnold ML (1998). Hybrid fitness in the Louisiana irises: analysis of parental and F1 performance. *Evolution* 52: 37-43.
- Chamberlain DF (1982). Revision of *Rhododendron* II. subgenus Hymenanthes. *Notes Roy Bot. Gard Edinb* 39: 209-486.
- Cross JR (1975). Biological flora of the British Isles: *Rhododendron ponticum* L. *J Ecol* 63: 345-364.
- Dobzansky T (1970). Genetics and the evolutionary process. New York: Columbia Univ. Press.
- Emms SK & Arnold ML (1997). The effect of habitat on parental and hybrid fitness: transplant experiments with Louisiana Irises. *Evolution* 51: 1112-1119.
- Güner A & Duman H (1998). A floristic excursion to Artvin and Camili. *Karaca Arboretum Magazine* 4: 55-68.
- Johnson JA, Wesselingh RA, Bouck AC, Donovan LA & Arnold ML (2001). Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana *Iris* hybrid population. *Mol Ecol* 10: 673-682.
- Kerner J (1895). Natural history of plants 2 (pp 588-9). London: Blackie and Son.
- Kyhos DW, Clark C & Thompson WC (1981). The hybrid nature of *Encelia laciniata* (Compositae: Heliantheae) and control of population composition by post-dispersal selection. *Syst Bot* 6: 399-411.
- Li Z, Pinson SRM, Paterson AH, Park WD & Stansel JW (1997). Genetics of hybrid sterility and hybrid breakdown in an interspecific rice (*Oryza sativa* L.) population. *Genetics* 145: 139-1148.
- Mallet J (1995). A species definition for the modern synthesis. *Trends Ecol. Evol.* 10: 294-299
- Milne RI, Abbott RJ, Wolff K & Chamberlain DF. (1999). Hybridization among sympatric species of *Rhododendron*: (Ericaceae) in Turkey: morphological and molecular evidence. *Am. J. Bot.* 86: 1776-1785.
- Milne RI, Terzioğlu S & Abbott RJ (2003). Maintenance of species barriers in sympatry: habitat-mediated selection against the second hybrid generation in *Rhododendron*. *Molecular Ecology* (in press).
- Rieseberg LH & Ellstrand NC (1993). What can molecular and morphological markers tell us about plant hybridization? *Crit Rev Pl Sci* 12: 213-241.
- Rieseberg LH & Wendel JF (1993). Introgression and its consequences in plants. Pp 70-109. In: Harrison R (ed). Hybrid zones and the evolutionary process. Oxford: Oxford Univ. Press.
- Rieseberg LH (1997). Hybrid origin of plant species. *Ann Rev Ecol Syst* 28: 359-389.
- Rieseberg LH & Carney SC (1998). Plant hybridisation. *New Phytol* 140: 599-624.
- Rieseberg LH, Archer MA & Wayne RK (1999). Transgressive segregation, adaptation, and speciation. *Heredity* 83: 363-372.
- Simons P (1988). The day of the Rhododendron. *New Sci* 119: 50-54.
- Stevens PF (1978). *Rhododendron* L. Pp 90-94. In: Davis PH (ed). Flora of Turkey and the East Aegean Islands, vol. 6. Edinburgh: Edinburgh Univ. Press.
- Wang H., McArthur ED, Sanderson SC, Graham JH & Freeman DC (1997). Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae). IV. Reciprocal transplant experiments. *Evolution* 51: 95-102.