Hybridization among sympatric species of *Rhododendron* (Ericaceae) in Turkey: morphological and molecular evidence[1]

Richard I. Milne,[2,4] Richard J. Abbott,[2] Kirsten Wolff,[2,5] and David F. Chamberlain[3]

[2]School of Environmental and Evolutionary Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK; and
[3]Royal Botanic Garden, Inverleith Row, Edinburgh EH20 3LR, UK

Rhododendron (Ericaceae) is a large genus in which barriers to hybridization are especially weak, but many species are maintained in sympatry. Hybridization among four species of *Rhododendron* subsect. *Pontica,* which occur in sympatry in Turkey, was investigated. Material of *R. ponticum, R. smirnovii, R. ungernii,* and *R. caucasicum* and their putative hybrids was collected from the wild. Based on morphology, chloroplast DNA and nuclear ribosomal DNA restriction fragment length polymorphism (RFLP) profiles, each accession was identified as a species or hybrid combination. Five of the six possible hybrid combinations among the four species were detected. *Rhododendron ponticum* × *R. smirnovii* was represented by a single individual and *R. caucasicum* × *R. smirnovii* by one small group of hybrid plants. The combinations *R. ponticum* × *R. ungernii* and *R. ungernii* × *R. smirnovii* showed evidence of frequent backcrossing, while *R. ponticum* × *R. caucasicum* appeared unusual in that an intermediate hybrid type was abundant, whereas hybrids with phenotypes approaching either parent were rare. Possible explanations of this latter situation are discussed. The results suggest that natural hybridization among *Rhododendron* species is common and that ecological factors are important in maintaining integrity when species occur in sympatry.

Key words: diploid hybrid speciation; ecological isolation; hybridization; introgression; *Rhododendron*; RFLP.

Although interspecific hybridization is recognized to be of major importance in plant evolution (Grant, 1981; Abbott, 1992; Arnold, 1992, 1997; Rieseberg and Wendel, 1993; Rieseberg, 1997; Rieseberg and Carney, 1998), spontaneous hybrid formation is not evenly distributed among plant taxa. Ellstrand, Whitkus, and Rieseberg (1996) have shown that the frequency of hybridization varies strongly among families, and whereas some plant genera contain large numbers of hybrids, the majority contain none. Genera prone to hybridization may sometimes contain many species occurring in sympatry without breakdown of species barriers (e.g., certain *Quercus* spp.—Whittemore and Schaal, 1991; Nason, Ellstrand, and Arnold, 1992; *Penstemon*—Wolfe and Elisens, 1994; Wolfe, Xiang, and Kephart, 1998). In such cases, the potential exists for the production of fertile hybrids, yet hybrid swarms rarely or never occur naturally, presumably because ecological factors limit hybrid formation or establishment. Possible mechanisms that limit hybridization in such genera may be those through which reproductive isolation evolved between some species pairs, and thus contributed to speciation and diversification within the genus as a whole. In this context, information regarding the frequency and nature of hybridization within a large genus is fundamental to understanding its history and how large numbers of potentially interfertile species might arise and be maintained.

With respect to woody plants, molecular markers have been used to investigate hybridization in wind-pollinated genera, notably *Quercus* (e.g., Whittemore and Schaal, 1991; Nason, Ellstrand, and Arnold, 1992; Jensen et al., 1993; Howard et al., 1997), and *Populus* (e.g., Paige and Capman, 1993; Rajora and Dancik, 1995), but less frequently among insect-pollinated woody species. That said, it has been shown that germplasm of one species may invade the range of another through long-distance pollen transfer in insect-pollinated *Aesculus* (dePamphilis and Wyatt, 1990) and *Eucalyptus* (Potts and Reid, 1988). Also, hybrids are frequent in island endemic groups such as the Hawaiian silversword alliance (Carr and Kyhos, 1986) and the Macaronesian genus *Argyranthemum* (Brochmann, 1987, and references therein). However, there is not as yet a large and widely distributed insect-pollinated woody genus in which the role of hybridization has been investigated in more than a few species.

Rhododendron (Ericaceae) is an example of a large woody genus in which hybridization may have played an important role in evolution and speciation. The very large number of horticultural hybrids in existence (over 1000; Bean, 1976) testifies to the weakness of genetic barriers towards hybridization in this genus, yet natural hybridization of rhododendrons has been little studied (Kron, Gawen, and Chase, 1993). *Rhododendron* subgenus *Hymenanthes* contains 225 species, which often occur in close sympatry; for example, 67 species occur in a small area of ~100 × 150 km in the eastern Himalayas (Chamberlain, 1982). Only 14 natural hybrids have been identified within subgenus *Hymenanthes* (Chamberlain, 1982), however the true extent of hybridization is almost

TABLE 1.   Localities in Turkey from which *Rhododendron* material was sampled.

Locality name (code)[a]	Longitude, latitude	Altitude (m)	Locality information and habitat	*Rhododendron* species present[b]
Artvin (Av)	41°07′N, 41°44′E	1800–2100	Hill ~10 km WSW of Artvin. Woodland, and grassland above treeline.	*R.p., R.c., R.s.,* plus two hybrids
Tiryal Daga (TD)	41°14′N, 41°36′E	1400–2400	Slope below Tiryal Daga mountains above Damar, 4 km SE of Murgul. Mainly *Rhododendron* scrub.	*R.p., R.u., R.s.,* and *R.c.,* plus four hybrids (see text).
Savval Tepe (ST)	41°11′N, 41°32–3′E	1700	10 km along a road running SW from Murgul below Gul Dagi. Woodland.	*R.p.* (common) *R.u.* (common)
Hopa (Ho)	41°24′N, 41°29′E	Sea level	By the road just south of Hopa. Shady undergrowth at roadside.	*R.p.* (occasional)
Camlihemsin (Ch)	41°01′N, 41°03′E	~1600	Very steep stream valley above Camlihemsin.	*R.p.* (abundant) *R.c.* (occasional) *R.c.* × *R.p.*
Ikisdere (Ik)	40°46′N, 40°35′E	1000	By main road (83) above Ikisdere; scrub between the river and the road.	*R.p.*
Ovit Daga, Soganli (Ov)	40°39′N, 40°43′E	~2000	By road from Ikisdere to Ispir, near Sirrikaya. Alpine grassland.	*R.c.*
N Turkey, Bolu (Bo)	40°43′N, 31°30′E	1000	12 km from Bolu, on main road (E5) to Duzce. Woods beside the road.	*R.p.* (abundant)
NW Turkey, Istranca	41°50′N, 27°41′E	800	By road from Demirkoy NW to Golyaka, understorey in woodland.	*R.p.* (local)
Daglari; Two sites (ID 1,2)	41°38′N, 28°06′E	50	Just above Kiyikoy, near coast; understory in woodland.	*R.p.* (local)

[a] Locality is in northeast Turkey unless stated otherwise. The code given represents each location in Fig. 1.

[b] The abbreviations *R.c., R.p., R.s.,* and *R.u.* represent *Rhododendron caucasicum, ponticum, smirnovii,* and *ungernii,* respectively.

certainly much greater in parts of the Himalayas where species boundaries appear incomplete. Actively speciating species complexes occur within this area (Argent et al., 1998) and in many cases clear morphological boundaries among species have not been determined. Partly because the taxonomy is so complex, it is not known to what extent hybridization has contributed to species diversity or intergradation of species in this region.

Smaller clusters of sympatric *Rhododendron* species occur elsewhere, which provide an opportunity to investigate the extent of natural hybridization within the genus. For example, in northeast Turkey and the adjacent Caucasus four species of subsection *Pontica,* subgenus *Hymenanthes* occur in sympatry, and although one hybrid (*R. ponticum* × *R. caucasicum*) occurs wherever the parents are sympatric, and two others are suspected (Chamberlain, 1982), these hybrids have not been well studied. An examination of the extent of hybridization among these four species would provide an indication of how frequently hybridization occurs among sympatric *Rhododendron* species throughout the range of the genus and would be a step towards understanding how species integrity is maintained.

The four species of *Rhododendron* subsection *Pontica* in northeast Turkey have different ecological preferences and altitude ranges. *Rhododendron ponticum* L. occurs from sea level to 1800 m (rarely to 2100 m) in forests (normally of *Fagus orientalis*; Stevens, 1978) or *Rhododendron* thickets (Chamberlain, 1982); *R. ungernii* Trautvetter occurs in forests from 1200 to 1850 m (Chamberlain, 1982), and appears to show the strongest requirement for shade (R. Milne, personal observations); *R. smirnovii* Trautvetter occurs in forests or scrub from 1500 to 2300 m, occasionally descending to 500 m (Chamberlain, 1982) with a preference for growing on rocky outcrops (R. Milne, personal observations). These

three species may occur together between 1200 and 1500 m, as at Tiryal Daga, near Murgul, northeast Turkey (R. Milne, personal observations). The fourth species, *R. caucasicum,* is found between 2000 and 3000 m in the open alpine zone of this area (Stevens, 1978), thus overlapping the range of *R. smirnovii* and to a much lesser extent *R. ponticum*; it also occasionally descends to altitudes as low as 1700 m, such as where late-lying snow patches indicate locally cold conditions (R. Milne, personal observations). Although hybrids are known to form between certain species pairs of this group, the four species are easily distinguished by four morphological characteristics, i.e., flower color, lower leaf surface indumentum, calyx lobe length, and ovary indumentum (see Table 2).

The most common hybrid is that between *R. ponticum* and *R. caucasicum* (*R.* × *sochadzeae* Charadze and Davlianidze), which is locally abundant between 1800 and 2300 m, and occurs sporadically at lower altitudes (Stevens, 1978; Chamberlain, 1982; Güner and Duman, 1998). This hybrid forms large colonies, and sometimes monocultures, between 1900 and 2100 m. Curiously, hybrid individuals are consistently intermediate between the parents in morphology (Stevens, 1978; Güner and Duman, 1998; R. Milne, personal observations), indicating that backcrosses probably are rare. The apparent absence of backcrosses has contributed to some uncertainty about whether *R.* × *sochadzeae* is indeed *R. ponticum* × *R. caucasicum* (Stevens, 1978). However, according to a cpDNA phylogeny of *Rhododendron* subsect. *Pontica* (Milne, 1997), *R. ponticum* and *R. caucasicum* are not sister species, and therefore *R.* × *sochadzeae* cannot be the progenitor of both species and, if a hybrid, must be the result of secondary contact.

Specimens of a putative hybrid between *R. smirnovii* and *R. caucasicum* have occasionally been observed, but their identity has not been confirmed (Chamberlain,

TABLE 2. Diagnostic morphological characteristics of *Rhododendron* species and putative hybrids found in Turkey.

		Character			
Presumed taxon	Code[a,b]	Flower color	Leaf below	Calyx lobe length (mm)	Ovary indumentum
R. caucasicum	C	White to cream	Compactly hairy	0–1	Brown, hairy
R. smirnovii	S	Pink	Woolly	0–1	White, hairy
R. ponticum (pure)	P	Pink	Glabrous	0–1	Glabrous
R. ungernii (white)	wU	White	Woolly	3–5	White, hairy
R. ungernii (pale pink)	rU	Pink	Woolly	3–5	White, hairy
R. ungernii × *R. smirnovii*	U×S	Pink	Woolly	1–5	White to tan, hairy
R. ponticum, possibly introgressed by *R. ungernii*	P(u)	Pink	Glabrous or nearly so	0–5	Variable
Putative *R. ponticum* × *R. ungernii*	P×U	Pink	Sparse hairs	3–5	Sparsely hairy
R. × *sochadzeae* (typical)	P×C	Pinkish white	Glabrous or nearly so	0–1	Usually hairy
R. caucasicum, pale pink flowers, possibly introgressed by *R. ponticum* or *R. smirnovii*	C(p/s)	Pinkish white	Compactly hairy	0–1	Brown, hairy
R. ponticum × *R. caucasicum* (hybrid swarm)	P×C	Pink to white	Variable	0–1	Glabrous to hairy
cf. *R. caucasicum* × *R. smirnovii*	C×S	Pale pink	Compact to woolly	0–1	Tan, hairy

[a] This letter code is used to represent plants of this morphological type in Table 5.
[b] Lowercase letters in parentheses indicate a species that has putatively contributed introgressed germplasm.

1982). Hybridization between *R. smirnovii* and *R. ungernii* also appears to occur (Stevens, 1978; Chamberlain, 1982), and introgression resulting from this is a possible cause of a polymorphism for flower color within *R. ungernii,* with individuals producing either white or pale-pink flowers, but again there is no confirmation of this. No hybrids have been recorded between *R. ponticum* and *R. smirnovii,* or between *R. ungernii* and either *R. ponticum* or *R. caucasicum.*

Kron, Gawen, and Chase (1993) demonstrated using morphological and cpDNA restriction fragment length polymorphism (RFLP) markers that hybridization and introgression had occurred between two species of *Rhododendron* (sect. *Pentanthera*) from Georgia, USA. Nuclear and cpDNA RFLP markers have been used by others to investigate possible instances of hybridization and/or introgression, for example among species of *Senecio* (Harris and Ingram, 1992; Comes and Abbott, 1999), *Orchis* (Caputo et al., 1997), *Stebbinocarpus* (Wallace and Jansen, 1995), and *Helianthus* (Rieseberg, Soltis, and Palmer, 1988; Rieseberg, Carter, and Zona, 1990). Chloroplast DNA markers are cytoplasmic and are thus not altered by recombination during backcrossing, and in many cases have provided evidence of introgression where nuclear molecular or morphological evidence of such has been lacking (Rieseberg and Wendel, 1993). In the current study, morphological, cpDNA, and nuclear rDNA markers have been used to identify *Rhododendron* individuals in northeast Turkey that contain germplasm of more than one species, allowing investigation of instances of hybridization and introgression among the four species of *Rhododendron* subsection *Pontica* that occur in this region.

MATERIALS AND METHODS

Desiccated leaf material (1 g of fresh leaf mass to ~25 g of coarse silica gel) and voucher specimens (in most cases two flowers and one leaf) were collected from *Rhododendron* plants in Turkey in June 1994 by R. Milne. In total, 120 accessions were gathered from seven sites in northeast Turkey plus three from north and northwest Turkey (Table 1; Figs. 1, 2). Plants were identified morphologically as *R. ponticum* (40 accessions), *R. caucasicum* (14 accessions), *R. smirnovii* (13 accessions), *R. ungernii* (nine white-flowered and seven pink-flowered accessions), putative derivatives of *R. ponticum* × *R. caucasicum* (26 accessions), and other putative hybrid derivatives (17 accessions). Accessions were referred initially to a species or putative hybrid combination according to the four principal morphological characteristics of corolla color, ventral leaf surface indumentum, calyx lobe length, and ovary indumentum (Table 2).

Seven of the accessions of *R. ponticum* were sampled from five sites in Turkey (Fig. 1, Table 1) where due to altitude or range differences no other *Rhododendron* species were present [i.e., Istranca Daglari (two sites; ID1 and ID2), Ikisdere (Ik), Bolu (Bo), and Hopa (Ho)]. Similarly, two accessions of *R. caucasicum* were sampled from one site, Ovit Daga (Ov) at Soganli pass. The remaining accessions were gathered from four sites in northeast Turkey (Fig. 2, Table 1): these were from a hill above Artvin (Av) (where *R. ponticum, R. smirnovii,* and *R. caucasicum* were present), Tiryal Daga (TD) above Murgul (where all four species were present), woods below Savval Tepe (ST) near Murgul (where *R. ponticum* and *R. ungernii* only were observed), and Camlihemsin (Ch), where *R. caucasicum* was present at 1700 m and formed a hybrid swarm, comprising a variety of hybrid phenotypes, with *R. ponticum.*
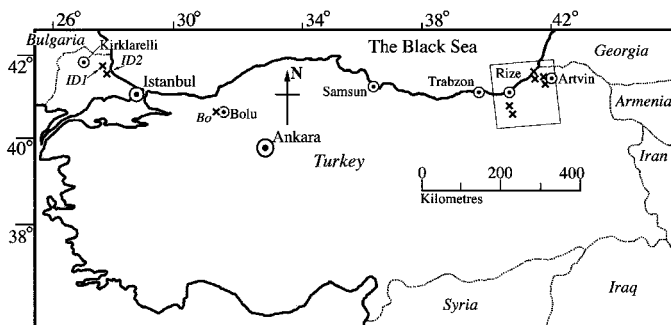

Fig. 1. Localities from which *Rhododendron* material was sampled in Turkey. Av—Artvin; Bo—Bolu; Ov—Ovit Daga; Ch—Camlihemsin; Sv—Savval Tepe; TD—Tiryal Daga; Ho—Hopa; ID1—Istranca Daglari (Demirkoy); ID2—Istranca Daglari (Kiyikoy); Ik—Ikisdere.
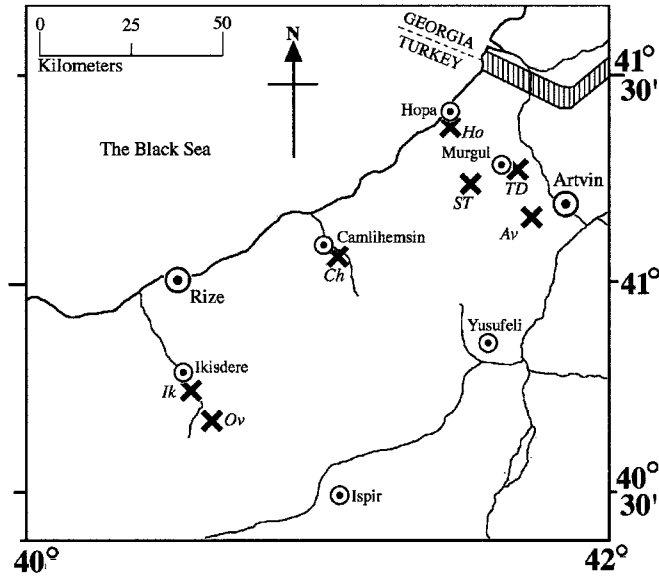
Fig. 2.   An enlargement of the area within the square indicated in Fig. 1.

Voucher specimens of material collected are stored in the Herbarium at R.B.G. Edinburgh.

Total DNA was extracted using the protocol of Whittemore and Schaal (1991), modified as follows: ground material in hexacyltrimethylammonium bromide (CTAB) buffer was warmed to 65°C and washed with 24:1 chloroform/isoamyl in place of methylene chloride; the extracts were treated with RNAse (1%, 20 μL/extract) for 1 h before precipitation; DNA was precipitated with two volumes of ice cold 96% ethanol. The sample was purified as follows: half a volume of 7.5 mol/ L sodium acetate was added, the mixture cooled for 20 min and centrifuged; the supernatant was precipitated with ethanol as above and rinsed with 76% alcohol/0.2 mol/L sodium acetate (twice) and 76% alcohol/10 mmol/L ammonium acetate (once), then resuspended in tris(hydroxymethyl)methylamine/ethylenediaminetetra-acetic acid (EDTA) buffer.

One accession of each species was selected for an initial RFLP survey to detect enzyme/probe combinations that might distinguish the cpDNA and rDNA of the four species. Material of *R. ponticum* was represented by a single accession from Istranca Daglari, northwest Turkey, while the other three species were represented by single accessions raised from wild seed collected in northeast Turkey. DNA extracts of these accessions were digested with 13 restriction enzymes to produce fragments that were separated by electrophoresis on 1% agarose gels. Following denaturation and neutralization, the fragments were transferred by Southern blotting to Electran (BDH) nylon membranes. Probe fragments were labeled with Digoxigenin (Boehringer Mannheim, Sussex, UK) according to the manufacturer's protocol. Membranes were hybridized with the labeled probes, washed and then detection was car-

ried out using Anti-Digoxigenin and CSPD (disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′chloro)tricyclo[3.3.1.1³,⁷]decan]-4-yl)phenyl phosphate) following the same protocol. Various combinations of the *Lactuca sativa* cpDNA probes (pLsC) described by Jansen and Palmer (1987) were employed. All 13 enzymes (*BamH*1, *Bcl*1, *Bgl2, Cla*1, *Dra*1, *EcoR*1, *Hae*3, *Hind*3, *Hpa*2, *Rsa*1, *Sal*1, *Sma*1, and *Stu*1) were used in conjunction with the combined pLsC probes 7, 9, 14, and 10, which are arranged contiguously in the cpDNA molecule (Jansen and Palmer, 1987). In addition, *Bcl*1, *Bgl2, Cla*1, *EcoR*1, *Hpa*2, *Sma*1, and *Stu*1 digests were probed with pLsC6; *Bcl*1, *Bgl2, Hpa*2, and *Stu*1 digests were probed with pLsC4; *BamH*1 and *Hpa*2 digests were probed with pLsC2; and *Bcl*1 and *Bgl2* digests were probed with the probes pLsC 5, 11, 12, and 13 combined. Thus, in the initial screen a total of 26 enzyme/probe combinations were employed. Membranes containing fragments of the four species, produced after digestion with each of the 13 restriction enzymes, were also probed with the *Triticum aestivum* rDNA probe pTa71, which is 9.1 kb in size (Gerlach and Bedbrook, 1979). After analyzing one accession of each species in this way, all of the remaining *Rhododendron* material was examined using only those enzyme/probe combinations that best distinguished the four species.

RESULTS

One enzyme/cpDNA probe combination, *Dra*1/pLsC7-10, generated RFLP profiles that were different in each of the four species, and therefore all accessions were examined using this enzyme/cpDNA probe combination. Other enzyme/cpDNA probe combinations were not used further. No additional cpDNA RFLP variation in *Dra*1/ pLsC7-10 restriction profiles was detected among all other accessions examined, and thus no infraspecific variation beyond that potentially caused by introgression was detected. Among the four species, nine different fragment sizes occurred in *Dra*1/pLsC7-10 RFLP profiles (Table 3), in addition to several very faintly visible fragments. The variation in these fragment sizes among species was too complex to be interpreted reliably in terms of specific mutations (Milne, 1997), and cpDNA haplotypes were therefore identified by the presence or absence of fragments (Table 3).

In regard to nuclear rDNA, only one enzyme, *Dra*1, produced RFLP profiles that allowed the four species to be distinguished from one another (Table 4). *Rhododendron ponticum* produced an rDNA RFLP profile that consisted of two unique fragments of 6.30 and 4.20 kb in length. The other three species all shared a fragment that was 5.49 kb in length; however a fragment of 4.71 kb in length was replaced by one of 4.51 kb in *R. ungernii*. The rDNA profile of *R. smirnovii* also included a unique fragment of 2.50 kb in length, which enabled it to be differentiated from that of *R. caucasicum*. Consequently, it was possible, in theory, to identify both parents of an

TABLE 3.   Chloroplast DNA restriction fragment profiles of four Turkish *Rhododendron* species following digestion of extracts with *Dra*1 and probing with plsC7-10. + indicates the presence of a band.

Rhododendron species	Code[a]	Fragment size (kb)								
		8.19	6.68	6.18	4.79	4.01	3.55	3.03	1.61	1.49
ponticum	P	−	+	−	−	−	+	+	+	−
caucasicum	C	−	+	+	−	−	+	−	+	+
ungernii	U	−	+	−	−	−	+	−	+	−
smirnovii	S	+	−	−	+	+	+	−	−	+

[a] This letter represents this species and RFLP profile in Table 5.

TABLE 4. Ribosomal DNA restriction fragment profiles produced following digestion of extracts with *Dra*1 and probing with pTa71. + indicates the presence of a band.

Rhododendron species	Code[a]	Fragment size (kb)					
		6.30	5.49	4.71[b]	4.51	4.20	2.50
ponticum	P	+	−	−	−	+	−
caucasicum	C	−	+	+	−	−	−
ungernii	U	−	+	−	+	−	−
smirnovii	S	−	+	+	−	−	+
ponticum + caucasicum[c]	P + C	+	+	+	−	+	−
ponticum + ungernii	P + U	+	+	−	+	+	−
ungernii + smirnovii	U + S	−	+	+	+	−	+
smirnovii + caucasicum	S + C	−	+	+	−	−	+

[a] These letters represent this RFLP profile in Table 5.

[b] This fragment is replaced by one of 4.84 kb in a specimen of *R. caucasicum* from R.B.G. Edinburgh.

[c] An identical fragment profile might also be produced by a derivative of *R. ponticum × R. smirnovii* if the 2.50-kb fragment is no longer visible (see text).

accession with an additive rDNA profile, except in the case of the additive profile of *R. caucasicum* and *R. smirnovii,* which was identical to pure *R. smirnovii.* Other than that which could be explained by introgression, no intraspecific variation was detected in the *Dra*1/pTa71 rDNA profiles of wild material of the four species; however, one accession of *R. caucasicum* in cultivation at R. B. G. Edinburgh had the 4.71 fragment replaced by a unique 4.84-kb fragment. Of 83 accessions that were identified as one species from their morphology, all possessed rDNA bands normal for that species; however, two possessed additional bands, and in both these cases introgression from a species that occurred in the vicinity provided a possible explanation. In addition, 27 accessions were found that possessed rDNA fragments of more than one species, which in 19 cases were of *R. ponticum* and *R. caucasicum.* The remaining 16 accessions displayed rDNA bands of just one species but were putatively identified as hybrids from their morphology.

Species and hybrid identification—On the basis of morphology, cpDNA type, and rDNA profile, all accessions were assigned to a species or derivative hybrid type (Table 5). *Rhododendron ponticum* was present at five localities where no other species was present, i.e., Hopa, Ikisdere, Bolu, and the two sites at Istranca Daglari (Table 5). Similarly, *R. caucasicum* occurred in the absence of other species at Ovit Daga. At the remaining sites more than one species was present. At Artvin, three species, *R. ponticum, R. caucasicum,* and *R. smirnovii,* were present, together with the hybrid *R. × sochadzeae* (*R. ponticum × R. caucasicum*). Also at this site was a single group of putative *R. caucasicum × R. smirnovii* hybrids. At Savval Tepe, *R. ponticum* and *R. ungernii* were present together in woodland. At Tiryal Daga all four species were present, together with *R. × sochadzeae,* which was abundant, plus the putative hybrids *R. ponticum × R. ungernii, R. ungernii × R. smirnovii* and *R. ponticum × R. smirnovii.* At both sites where it was present, white- and pink-flowered accessions of *R. ungernii* occurred in approximately equal numbers. At Camlihemsin, a small number of accessions of *R. caucasicum* was present at 1600 m (well below its usual altitude range) together with *R. ponticum,* which was abundant in this vicinity; a large number of putative hybrid derivatives between these two

species with highly variable morphology was found at this site.

Rhododendron ungernii × R. smirnovii—Among the four pink-flowered accessions of *R. ungernii* examined from Tiryal Daga, three possessed an additive rDNA profile with *R. smirnovii* (Table 5). In contrast, none of the white-flowered accessions of *R. ungernii* examined possessed molecular markers from *R. smirnovii.* Another accession from this area had the cpDNA of *R. ungernii,* the short calyx lobes of *R. smirnovii,* rDNA fragments of both species, and leaf indumentum of intermediate color, and hence may represent a derivative intermediate between the parents, and possibly an F_1. These findings indicate that hybridization is occurring between *R. ungernii* and *R. smirnovii* at this site and that backcrossing occurs towards *R. ungernii.* At Savval Tepe, where *R. smirnovii* was not observed, none of the four pink-flowered accessions of *R. ungernii* examined possessed the *R. smirnovii* rDNA marker; however, it is possible that in this case introgression occurred many generations ago and that the *R. smirnovii* rDNA marker has been lost through concerted evolution or repeated backcrossing to *R. ungernii.* The results obtained show that introgression from *R. smirnovii* is linked to pink flower color in *R. ungernii* in at least some populations and provide support for a hypothesis that gene flow from *R. smirnovii* is the cause of the flower color polymorphism in *R. ungernii.*

Rhododendron ungernii × R. ponticum—Two accessions from Tiryal Daga possessed the cpDNA of *R. ponticum* and an additive rDNA profile of *R. ponticum* and *R. ungernii* and appear to represent the first records of a hybrid between these two species. These had corollas a lighter shade of pink than *R. ponticum,* sparsely white-hairy ovaries, long calyx lobes similar in size to typical *R. ungernii,* and very small crisped hairs on the leaf underside (Table 2). They occurred at ~1600 and 1750 m, respectively, in *Rhododendron* scrub where *R. ungernii* and *R. ponticum* were common and *R. smirnovii* occasional. A third accession from this area also had an additive rDNA profile of the two species, but was closer to *R. ponticum* in morphology (specifically, the corolla color was similar to pure *R. ponticum* and the calyx lobes were less prominent). Six other accessions from the vicinity

TABLE 5. Categorization of accessions according to their morphological, cpDNA, and rDNA characteristics. The letters P, C, U, and S indicate *Rhododendron ponticum, caucasicum, ungernii,* and *smirnovii,* respectively.

Rhododendron species/hybrid	Locality	No. of plants	Morphology[a]	cpDNA[b]	rDNA[c]	Species in ancestry[d]
R. ponticum (far from other species)	Istranca	3	P	P	P	P only
	Bolu	1	P	P	P	P only
	Ikisdere	2	P	P	P	P only
	Hopa	1	P	P	P	P only
R. ponticum	Camlihemsin	7	P	P	P	P only
	Artvin area	7	P	P	P	P only
	Tiryal Daga	17	P	P	P	P only
	Savval Tepe	2	P	P	P	P only
R. caucasicum (far from other species)	Ovit Daga	2	C	C	C	C only
R. caucasicum	Camlihemsin	3	C	C	C	C only
	Artvin	7	C	C	C	C only
	Tiryal Daga	2	C	C	C	C only
R. smirnovii	Artvin area	7	S	S	S	S only
	Tiryal Daga	6	S	S	S	S only
R. ungernii (white flowers)	Savval Tepe	2	wU	U	U	U only
	Tiryal Daga	6	wU	U	U	U only
	Tiryal Daga	1	wU	P	U+P	U and P
R. ungernii (pale pink flowers)	Savval Tepe	4	rU	U	U	U only
	Tiryal Daga	3	rU	U	S+U	U and S
R. ungernii (deep pink flowers)	Tiryal Daga	1	rU	U	U	U only?
R. caucasicum (pale pink flowers)	Artvin	2	C(p/s)	C	C	C(+P/S?)
R. × sochadzeae (large populations)	Tiryal Daga	3	Z	C	P+C	C and P
	Artvin	2	Z	C	C	C and P
	Artvin	8	Z	C	P+C	C and P
R. × sochadzeae (close to large populations)	Tiryal Daga	1	Z	C	C	C(+ P?)
	Tiryal Daga	1	Z	P	P+C	C and P
	Artvin	2	P	P	P+C	P and C
	Artvin	1	Z	C	P+C	C and P
R. ponticum × R. caucasicum: hybrid swarm						
R.p. × R.c. (intermediate)	Camlihemsin	1	P×C	P	P+C	C and P
R.p. × R.c. (intermediate)	Camlihemsin	2	P×C	C	P+C	C and P
R.p. × R.c. (nearer *R. ponticum*)	Camlihemsin	1	P×C	C	C	C and P
R.p. × R.c. (nearer *R. caucasicum*)	Camlihemsin	1	P×C	C	P+C	C and P
R.p. × R.c. (nearer *R. caucasicum*)	Camlihemsin	1	P×C	C	P+C	C and P
R. ponticum, introgressed by *R. ungernii*?	Tiryal Daga	1	P(u)	P	P+U	P and U
	Tiryal Daga	6	P(u)	P	P	P (+ U?)
R. ponticum × R. ungernii	Tiryal Daga	2	P×U	P	P+U	U and P
R. ungernii × R. smirnovii	Tiryal Daga	1	U×S?	U	S+U	U and S
R. caucasicum × R. smirnovii	Artvin	3	C×S	C	S	C and S
	Artvin	2	C×S	S	S	C and S
Unidentified	Tiryal daga	1	?	S	P+C[e]	P and S

[a] See Table 2 for morphological characters indicated by code.
[b] See Table 3 for cpDNA RFLP profile indicated by code.
[c] See Table 4 for rDNA RFLP profile indicated by code.
[d] Accessions for which a second species' involvement is indicated by morphology but not molecular data and are unconfirmed hybrids, labeled with a question mark.
[e] See footnote c, Table 4.

possessed sparse ovary hair and/or long calyx lobes, but matched pure *R. ponticum* in other morphological and molecular characteristics; many other accessions were observed with similar characteristics but were not subjected to molecular examination. One accession of *R. ungernii* was found in this area that had white flowers and showed no morphological evidence of introgression, but had the cpDNA of *R. ponticum* and an additive rDNA profile of both species. Thus it appears that bidirectional introgression occurs following F₁ production between these two species and that backcrosses towards *R. ponticum* are considerably more numerous than F₁s. These results do not suggest that introgression from *R. ponticum* has contributed to the flower color polymorphism in *R. ungernii*. However, as only one putative backcross to *R.*

ungernii was detected, the results do not provide strong evidence against the possibility.

Rhododendron ponticum × R. smirnovii—One accession, present at ~1400 m in the Tiryal Daga area, appeared to represent a hybrid derivative of *R. ponticum* and *R. smirnovii* (Table 5). It had short calyx lobes, a pubescent ovary, and sparsely pubescent ventral leaf surface, but all corollas had fallen. The accession had the cpDNA of *R. smirnovii* and expressed four rDNA bands, of which two were those unique to *R. ponticum* and two (4.71 and 5.49 kb) were common to *R. smirnovii* and *R. caucasicum*. As the involvement of *R. smirnovii* is proved by the cpDNA profile and the accession was collected 600 m below the normal range of *R. caucasicum* and 300

m below that of *R. × sochadzeae,* the accession must be a derivative involving *R. ponticum* and *R. smirnovii,* and the additional involvement of *R. caucasicum* is unlikely. Therefore, the rDNA profile observed is likely to be an additive profile of *R. ponticum* and *R. smirnovii* minus the distinctive 2.50-kb fragment of the latter (Table 4).

Rhododendron caucasicum × R. smirnovii—*Rhododendron smirnovii* and *R. caucasicum* occur together on the hill above Artvin, and one small group of putative hybrids, intermediate in morphology between the two species, was present at this site. All five individuals examined had an rDNA profile that could be *R. smirnovii,* or *R. smirnovii* plus *R. caucasicum*; however three accessions had the cpDNA of *R. caucasicum* and are thus shown to be hybrids by the molecular data (Table 5). The other two accessions had the cpDNA of *R. smirnovii,* but their morphological similarity to the other two accessions strongly indicates that these had a similar hybrid origin. Therefore it appears that either one of these two species can act as the cpDNA donor in this hybrid derivative. No firm evidence of backcrossing in either direction was detected, although two accessions of *R. caucasicum* with pale-pink flowers may have been backcrosses from *R. smirnovii,* but no cpDNA or rDNA evidence of such was detected (Table 5).

Rhododendron ponticum × R. caucasicum (= R. × sochadzeae)—The hybrid between *R. ponticum* and *R. caucasicum, R. × sochadzeae,* was plentiful on the hill above Artvin, mixed with similar numbers of *R. caucasicum* and *R. smirnovii.* It was also common at Tiryal Daga where it occurred in virtual monoculture on slopes between 1900 and 2100 m, although not on the steeper or rockier slopes, where it was replaced by *R. smirnovii.* Of 13 accessions sampled from these locations, all had the cpDNA of *R. caucasicum* and 11 had an additive rDNA profile of *R. caucasicum* and *R. ponticum*; the remaining two expressed only the rDNA bands of *R. caucasicum* (Table 5). Three accessions of *R. × sochadzeae* were collected from lower altitudes, among populations of *R. ponticum,* and these were more variable in their molecular characteristics. One had the cpDNA of *R. ponticum* and an additive rDNA profile of *R. ponticum* and *R. caucasicum,* a second had the same additive rDNA profile and the cpDNA of *R. caucasicum,* and the third had the molecular characteristics of *R. caucasicum* alone. In addition, two accessions of morphologically typical *R. ponticum* at Artvin, and one at Camlihemsin, all in close proximity to accessions of *R. × sochadzeae*, showed evidence of nuclear introgression in that they expressed the rDNA bands of *R. caucasicum.* Two accessions were found at Artvin that matched *R. caucasicum* in molecular and morphological characterization, except for having very pale-pink flowers. These accessions may have been backcrosses towards *R. caucasicum* from *R. × sochadzeae,* or from *R. smirnovii* as suggested above.

Six hybrid accessions from the hybrid zone at Camlihemsin were examined, of which five had the cpDNA of *R. caucasicum* and one had the cpDNA of *R. ponticum.* Five accessions possessed the additive rDNA profile of the two species, but one, which resembled *R. ponticum* more closely in morphology, expressed only the rDNA bands of *R. caucasicum.*

DISCUSSION

Of the six possible hybrid combinations between the four species of *Rhododendron* subsection *Pontica* that occur in Turkey, derivatives of five were identified. The only hybrid combination for which no evidence was found was between *R. ungernii* and *R. caucasicum.* Hybrids between these two white-flowered species might have been overlooked. However, they were the only two species not observed growing close together at any site visited. The fact that such hybrids are absent or very rare may be due to both ecological differences and limited contact between the two parents. In regard to the other hybrid combinations, in one case (*R. × sochadzeae*) the intermediate phenotypes were abundant, while in two other cases (*R. ungernii × R. smirnovii* and *R. ungernii × R. ponticum*) putative F_1s were rare, whereas backcrosses were relatively frequent. This latter situation appears to resemble that reported by Arnold et al. (1993) for Louisiana irises in which F_1 formation was rare although later generation hybrid derivatives were common. In the fourth case (*R. smirnovii × R. caucasicum*), a single small group of putative F_1 individuals was detected, whereas in the final case (*R. smirnovii × R. ponticum*) only a single individual hybrid derivative was found. In these last two cases it was not certain whether backcrosses might be formed but were not detected, or whether the F_1s do not, or only rarely, generate progeny.

The present study has also documented examples of nuclear without cytoplasmic introgression (*R. caucasicum* into *R. ponticum*; *R. smirnovii* into *R. ungernii*) and of both nuclear introgression and plastid transfer without apparent morphological introgression (*R. ponticum* into *R. ungernii*). An apparent example also occurred of the loss of part of one parent's rDNA profile in a hybrid derivative: the putative *R. ponticum × R. smirnovii* individual had both rDNA fragments of *R. ponticum* but just two of the three fragments characteristic of *R. smirnovii.* The loss of all rDNA fragments from one species from the profile of an F_1 has been observed in *Zea* (Zimmer, Jupe, and Walbot, 1988), *Avena* (Fabijanski et al., 1990), and *Senecio cambrensis* (Harris and Ingram, 1992), and could result from concerted evolution. However, the loss of part of a profile would appear to require either recombination within a hybrid or rDNA polymorphism within *R. smirnovii.*

The four *Rhododendron* species investigated occur together in the Tiryal Daga (northeast Turkey) area, where all of the hybrid combinations mentioned except *R. smirnovii × R. caucasicum* were observed. All four species are abundant at this site and despite the evidence of gene flow between them, in the field the great majority of plants could easily be referred to one species. The site appears to have been subject to human disturbance, and much of the *Rhododendron* scrub here may result from tree felling. This may have reduced the effect of habitat preferences as barriers to contact and hybridization between species. In contrast, *R. ponticum* and *R. ungernii* occur side by side in an undisturbed woodland at Savval Tepe and here no hybrids were found.

In general, the morphology of *R. × sochadzeae* was observed to be remarkably consistent at Artvin and Tiryal Daga, which concurs with the observations of Stevens (1978) and Güner and Duman (1998). Accessions whose morphology indicated a backcross from *R. × sochadzeae* to either parent were very rare compared to accessions with the intermediate morphology typical of large populations of *R. × sochadzeae.* The consistent morphology of this hybrid would suggest that segregation of morphological characteristics is not occurring. In contrast, at Camlihemsin a very different situation existed. Here a small number of individuals of *R. caucasicum* occurred at ~1600 m in a northeast-facing valley, which was both very steeply sloping and steep-sided and in which snow patches persisted until at least late June; there was no sign of recent human habitat disturbance in this valley. *Rhododendron ponticum* grew abundantly on the sides of the valley, and hybrid derivatives of the two species were more common than *R. caucasicum* with which they occurred in the valley bottom. In this case, the intermediate phenotype did not predominate among the hybrids and a gradation of colors from creamy white to magenta was observed. Also there was no altitudinal zonation of the two species and their hybrids as there was at Tiryal Daga.

Clearly the limited molecular results presented here do not answer the question of why the morphology of the *R. × sochadzeae* hybrid in some populations appears constant. Clonal reproduction has been reported in some *Rhododendron* species, for example *R. ferrugineum,* a distantly related species that occurs at similar altitudes in the European Alps (Escaravage et al., 1998), and could account therefore for some of the phenotypic uniformity observed in *R. × sochadzeae.* However, it is unlikely to be the sole explanation, because even if reproduction is predominately clonal at sites like Tiryal Daga, there must be some recruitment from seed, and as the presence of backcrosses at Camlihemsin indicates that the F_1 is fertile, the problem of why only F_1s or phenotypically intermediate individuals are recruited is not circumvented.

Another possible explanation is that the large populations are polyploid and behave as a species while the hybrid zone at Camlihemsin is homoploid. However, an accession grown from seed collected by R. Milne from the center of a large *R. × sochadzeae* population at Tiryal Daga was found to have the same chromosome number, $2n = 26$, as both parents (Dr. H. McAllister, University of Liverpool, personal communication). Furthermore, no other polyploids are known within subgenus *Hymenanthes,* and *R. × sochadzeae* has never been recorded outside of the ranges of its two parents, which one might expect were it an independent polyploid species. Alternatively, some factor may make backcrosses rare in the presence of large numbers of F_1s; one possibility is that flower-constant pollinators may be unlikely to transfer pollen between the hybrid and its parent species (Rieseberg and Wendel, 1993; Wolfe, Xiang, and Kephart, 1998). Also, selection against backcrosses has occasionally been observed in other plant species (Keim et al., 1989; Bert and Arnold, 1995; Allan, Clark, and Rieseberg, 1997), and this may occur in *R. × sochadzeae.* Such ecological selection may have led to speciation in the putative diploid hybrid species *Encelia virginensis* (Allan, Clark, and Rieseberg, 1997), which differs from *R.*

× sochadzeae in that it now occurs allopatrically from its parents. A possible hypothesis is that *R. × sochadzeae* benefits from having a set of coadaptive genes from each parent, which confer hardiness to higher altitudes (*R. caucasicum*) and competitiveness at lower altitudes (*R. ponticum*) and only plants that are genetically intermediate contain both complete sets of these genes. If backcrossing were rare and most hybrid derivative individuals arose from crosses between intermediates rather than recruitment of new F_1s, then the effects of segregation would disappear through several generations, as is the case in stabilized hybrid derivatives (e.g., Arnold, 1993; Urbanska et al., 1997). Whatever mechanism limits backcross formation at Tiryal Daga and Artvin, it has clearly broken down at Camlihemsin, possibly because *R. caucasicum* and *R. × sochadzeae* are present in relatively small numbers there. If *R. × sochadzeae* is preferentially pollinated by *R. × sochadzeae* pollen, then this might limit backcrossing where it is abundant but not where it is greatly outnumbered by *R. ponticum,* as at Camlihemsin. A comparable situation exists between two salamander races, which do not normally breed where their ranges meet but formed a hybrid swarm where an outlier of one race was surrounded by greater numbers of the other (Wake, Yanev, and Frelow, 1989). There may be some parallels between *R. × sochadzeae* and *Rhododendron × intermedium* (*R. ferrugineum × R. hirsutum*), which is rare at some sites where parents co-occur, but abundant at others (Grant, 1981); however this hybrid does not appear to have been observed or studied in detail.

The nature of *R. × sochadzeae* as a taxon remains open to question. Although the molecular results here confirm that it is the hybrid between *R. ponticum* and *R. caucasicum,* the morphological consistency of *R. × sochadzeae* suggests that it is a stabilized hybrid derivative. However, it occurs in the vicinity of both parents, and accessions with more variable molecular characteristics were observed outside of the main *R. × sochadzeae* population. This indicates that hybridization continues to occur between the parent species, and there is no reason to assume these hybrids are not interfertile with the other *R. × sochadzeae* plants. *Rhododendron × sochadzeae* may be an entity that combines the beneficial traits of both parents, maintains a degree of phenotypic consistency through selection against backcrosses and extreme segregants, and retains an unusually high genetic diversity through periodic recruitment of F_1s. Further investigation of the genetics of *R. × sochadzeae* could be highly informative in regard to studies of interactions between fertile hybrid populations and their parents, and hence the mechanisms underlying hybrid speciation.

The evidence of this study indicates that hybrid formation between sympatric species of *Rhododendron* is likely to be common, and for closely related species may be the rule rather than the exception. As the four species in this study belong to the same subsection (*Pontica,* subgenus *Hymenanthes*), the results do not necessarily indicate whether less closely related species of *Rhododendron* form hybrids as frequently as these species. From these results, however, it is reasonable to assume that hybridization is probably fairly frequent between sympatric species of subgenus *Hymenanthes,* particularly in the Himalaya region where such species are most con-

centrated and, for example, many species of the large subsection *Taliensa* have been recorded within one small area (Chamberlain, 1982). It would be of interest, therefore, to determine how such species are maintained in sympatry despite interspecific gene flow and whether hybridization has been a significant factor in the evolution of the large number of species within the genus *Rhododendron.*

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