Parallel Evolution in Lichen-Forming Fungi

Abstract. *The asexual lichen-forming fungus* Parmelia hypotropa *has two common chemical races that differ sharply in biogeography and niche characteristics and appear to have been derived by morphologic parallelism from chemically identical races of the closely related* Parmelia perforata, *which is sexual. Among the lichen fungi many chemically variable asexual morphs, which conventional taxonomies treat as species, are probably polyphyletic like* Parmelia hypotropa.

Lichen-forming fungi are notable for the large array of unique secondary products that they sythesize and accumulate (*1*). Although most species of these fungi are qualitatively uniform in their secondary chemistries, hundreds of the major morphs (morphological types) within genera have two or more chemical races (*2*). Most lichenologists consider chemically different races of uniform morphology to be conspecific and to have been derived subsequent to the morphological differentiation (*3*). We can now give evidence that one common chemically variable morph, *Parmelia hypotropa*, is the product of parallelism from separate chemical lines and that the species which this morph is considered to represent is polyphyletic. If this evolutionary phenomenon is as widespread as the abundance of analogous chemically variable morphs would suggest, then the presently accepted view of the origin of many well-known "species" of lichen-forming fungi is wrong.

The closely related *Parmelia perforata* (Jacq.) Ach. and *P. hypotropa* Nyl. are two of the commonest foliose lichens epiphytic on trees in the eastern United States. The first morph lacks asexual propagules and is usually fertile (*4*), bearing apothecia with spores, the ultimate product of the sexual process (Fig. 1A). The second morph is almost exclusively sterile (*4*) and reproduces by easily shed soredia (one or several algal cells with attached hyphal fragments) produced in powdery masses on the margins of the lobes (Fig. 1D). Both morphs have been reported (*5*) to produce norstictic acid, a β-orcinol depsidone that is not *O*-methylated (a compound type unique to lichen fungi). Additionally, the sorediate-sterile morph has been believed to be consistent in the production of stictic acid, an *O*-methylated derivative of norstictic acid. We have used a new, standardized, highly sensitive method (*6*) of thin-layer chromatography involving three solvent systems to analyze the 863 specimens of this species group in the Smithsonian Institution, Washington, D.C., and the herbarium at Duke University. We found that each morph has two chemical races with distinctive geographic distributions.

Individuals belong to one or the other of two chemical types. They produced in the medulla either norstictic acid and connorstictic acid (a related depsidone of unknown structure) or stictic acid, constictic acid (both *O*-methylated β-orcinol depsidones), and norstictic acid. (All individuals also produced the β-orcinol *para*-depside atranorin in the upper cortex.) Maps of the provenance of the tested specimens show that most of the esorediate-fertile individuals belong to a widespread norstictic-connorstictic race (Fig. 1A), but that a few belong to a rare and previously unsuspected stictic-constictic-norstictic race localized in the coastal plain in Texas and Louisiana (Fig. 1B). The chemical races of the sorediate-sterile morph are largely allopatric in the East. The norstictic-connorstictic race is broadly distributed there, but is absent from the lower southeastern coastal plain (Fig. 1C). The stictic-constictic-norstictic race is almost exclusively confined to the Atlantic coastal plain from Cape Cod, Massachusetts, to southern Mississippi (Fig. 1D).

It was previously thought that all sorediate-sterile individuals of this complex produced stictic acid and that the failure to detect this substance in many specimens was due to an insensitivity of the now passé microcrystallographic analytic technique (*5*). But an appreciation of the fact that individuals

Table 1. Analysis of the association of chemical races and bark substrates in 520 randomly selected specimens. The norstictic-connorstictic race is designated by nor, the stictic-constictic-norstictic race by sti. The percent probability *P* of the observed association resulting from chance is given (*11*); values of *P* greater than 5 percent are not significant.

Distance from coast (km)	Location	Race	Number on		*P* (%)
			Conifers	Hardwoods	
	Stands less than 10 km from the coast				
1	Edenton, Chowan Co., North Carolina	nor	1	11	
		sti	19	9	0.1
5	Accomack, Accomack Co., Virginia	nor	1	6	
		sti	19	14	9.1
7	Nixonton, Pasquotank Co., North Carolina	nor	0	2	
		sti	20	18	48.7
	Pooled	nor	2	19	
		sti	58	41	0.006
	Stands 10 to 40 km from the coast				
34	Bacons Castle, Surry Co., Virginia	nor	7	16	
		sti	13	4	0.9
37	Isle of Wight, Isle of Wight Co., Virginia	nor	12	17	
		sti	8	3	15.5
	Pooled	nor	19	33	
		sti	21	7	0.2
	Stands 41 to 60 km from the coast				
55	Savedge, Surry Co., Virginia	nor	12	20	
		sti	8	0	0.3
55	Spring Grove, Surry Co., Virginia	nor	18	19	
		sti	2	1	> 99
56	Carrsville, Isle of Wight Co., Virginia	nor	15	20	
		sti	5	0	4.7
60	Ivor, Southampton Co., Virginia	nor	15	19	
		sti	5	1	18.2
	Pooled	nor	60	78	
		sti	20	2	0.004
	Stands 61 to 120 km from the coast				
97	Drewyville, Southampton Co., Virginia	nor	19	20	
		sti	1	0	> 99
97	Branchville, Southampton Co., Virginia	nor	18	19	
		sti	2	1	> 99
104	Leggett, Edgecombe Co., North Carolina	nor	20	20	
		sti	0	0	
120	Emporia, Greenville Co., Virginia	nor	19	20	
		sti	1	0	> 99
	Pooled (Virginia)	nor	56	59	
		sti	4	1	36.4

of both morphs belong to one of two distinct chemical races with different geographic ranges leads to the question of whether the asexual morph (*P. hypotropa*) evolved from the esorediate-fertile counterpart (*P. perforata*) and then differentiated chemically (the currently accepted notion for chemically variable morphs of this type), or whether the two chemical races of the asexual morph were derived independently from the corresponding chemical races of the sexual morph. We thought that a clue might come from evaluating whether the ranges of the chemical races of morphs result from disparate ecologies. (In other words, do the races differ in something more than the chemistry of the secondary natural products?) The experiments that immediately suggest themselves are unfeasible because of immense technical problems in culturing lichens. Still, an approach to the problem can be made in the herbarium.

In analyzing museum specimens we noted that the races of the sorediate-sterile morph seemed to occur disproportionately on the barks of various trees. Epiphytic cryptogams are extremely sensitive to the physical and chemical characteristics of the bark on which they grow, and a classic dichotomy in the ecology of these organisms occurs on conifers and hardwoods (*5*)—the generally softer, more acidic, resinous bark of the former compared to the generally harder, less acidic, nonresinous bark of the latter. Although many cryptogamic epiphytes occur occasionally on both conifers and hardwoods, none are known to have tolerances so wide that they occur with equal abundance on a broad range of trees of both types. We tallied the substrates of all specimens studied from the eastern United States for which the labels gave the information.

The 299 specimens of the norstictic-connorstictic race of the sorediate-sterile morph showed a high association with hardwoods (79 percent). The norstictic-connorstictic race was only half as well represented from conifers (21 percent of the specimens) as the stictic-constictic-norstictic race (40 percent). Is the norstictic-connorstictic race better adapted to life on hardwoods and the stictic-constictic-norstictic race better adapted to life on conifers? The herbarium data would seem to say yes, but it could be argued that individuals of the norstictic-connorstictic race range through the east-central states (Fig. 1C), where the vegetation is dominated by hardwoods, and that those of the stictic-constictic-norstictic race range through the Atlantic coastal plain (Fig. 1D), where much of the vegetation is dominated by pines. The apparent substrate relationships might then reflect only the type of forest trees prevalent in the main range of each race.

To eliminate the variable of geography we analyzed the habitat ecology of the chemical races of the sorediate-sterile morph where these grow together. In the region of apparent sympatry we found that the races actually occur together commonly only in southeastern Virginia and northeastern North Carolina. We selected there 13 woodland stands of approximately equal mixtures of conifers (mostly *Pinus taeda*) and hardwoods (such as *Acer rubrum*, *Liquidambar styraciflua*, and *Quercus* spp.). Trunks were examined at the height of 1.3 m until two sorediate specimens had been taken from each of ten conifers and ten hardwoods.

Analysis of the 520 randomly selected individuals (Table 1) confirms the theory suggested on the basis of herbarium data. The chemical races of

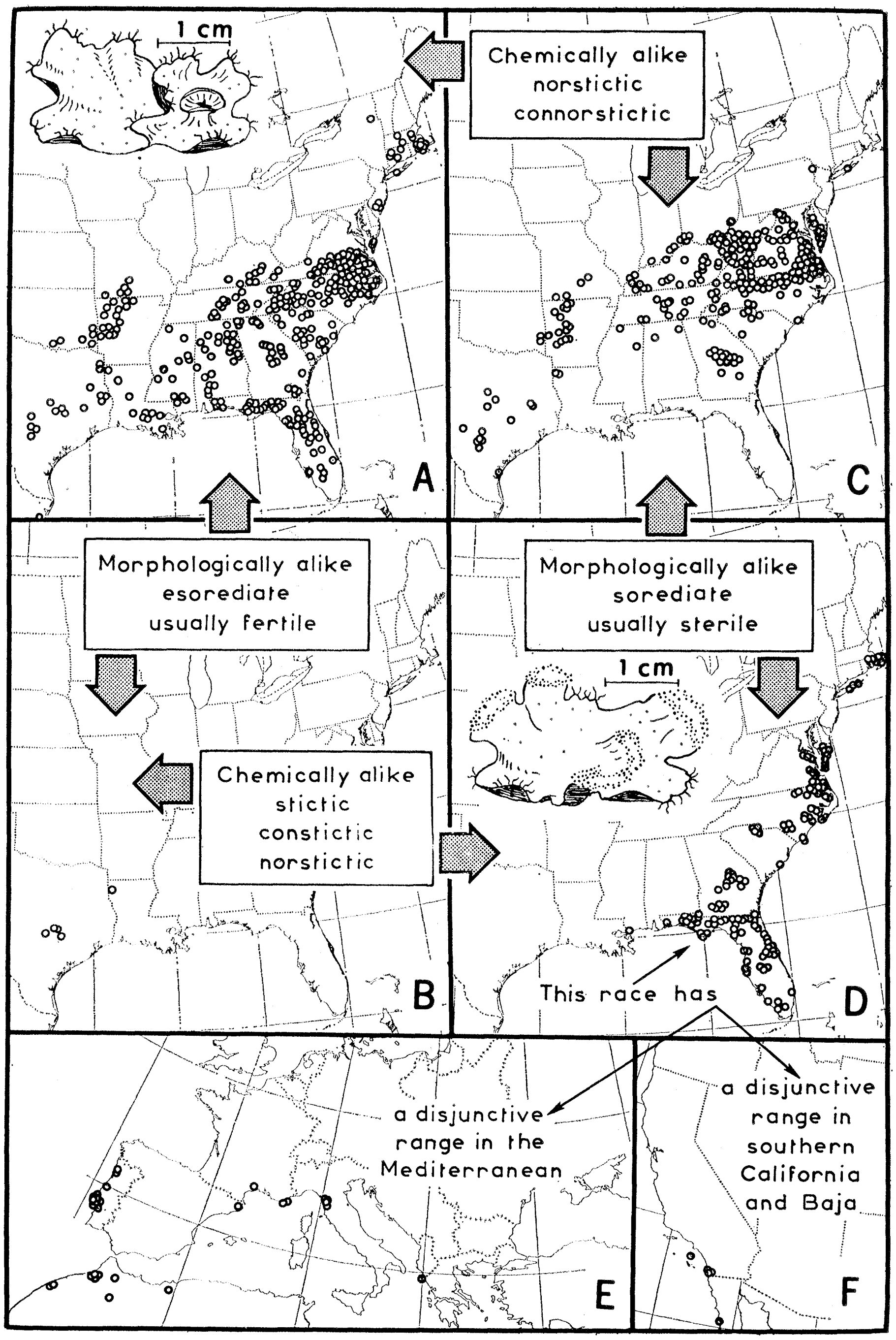

Fig. 1. Ranges of the chemical races of the two morphs. (A and B) Ranges of the races of the esorediate-fertile morph (*Parmelia perforata*). (C to F) Ranges of the races of the sorediate-sterile morph (*P. hypotropa*).

the sorediate-sterile morph do indeed have different niche characteristics. In every stand where both chemical races were present the norstictic-connorstictic race was encountered more frequently on hardwoods than on conifers, and the stictic-constictic-norstictic race was encountered more frequently on conifers than on hardwoods. At some localities these associations were statistically not significant, but in every instance the trend is in the same direction. However, when the data from stands geographically near each other are pooled (thereby yielding a sample of larger size), the pooled data are significant at the 1 percent or 0.1 percent level. (The only exception is for stands more than 90 km from the coast, where the stictic-constictic-norstictic race becomes rare and the number of encounters in samples was low.) The pooled data and the maps (Fig. 1, C and D) show the overriding effect of gross climatic change from the seaboard to the interior. The complex of environmental parameters that makes up the east-west climatic gradient in the mid-Atlantic states causes an abrupt replacement inland of the coastal stictic-constictic-norstictic race by the norstictic-connorstictic race. At 200 km west of the Atlantic coast the stictic-constictic-norstictic race of the sterile morph is eliminated, but the norstictic-connorstictic race of that morph ranges another 1400 km westward through Missouri (*7*). The dependence of the stictic-constictic-norstictic race of the asexual morph on near-coastal environments is further seen in the disjunctive representation of this race in the Mediterranean region (Fig. 1E) and in southern California and Baja California (Fig. 1F) (*8*).

The norstictic-connorstictic race of the asexual morph must have been derived directly from the corresponding chemical race of its fertile counterpart by the production of soredia and the subsequent suppression of sexuality. The geographic ranges of the races are virtually identical with the exception of the absence of the norstictic-connorstictic race from the Gulf coastal plain. More important, the ecologies of both races are virtually identical—both are highly adapted to life as epiphytes of hardwoods. Although no field studies were made on the norstictic-connorstictic race of the esorediate-fertile morph, 94 percent (254) of the 269 herbarium specimens of this race seen with appropriate substrate data came from hardwoods. Likewise, the ecologically different stictic-constictic-norstictic race of the asexual morph must have evolved from the fertile but chemically identical race now known from only Texas and Louisiana (*9*).

The suggestion that in lichen fungi the chemically variable asexual morphs, which conventional taxonomies treat as species, may be polyphyletic and the product of parallel evolution is new. Poelt (*10*) put forth this theory only this year, by using examples with hypothetical but plausible extinct sexual ancestors to explain certain extant asexual morphs. Many of the commonest lichens are known only as chemically variable asexual morphs, and few asexual-sexual morph pairs coexist today. The research reported here provides the first example of a full set of chemical races of sexual and asexual morphs of which the origin of the latter is better explained by parallelism from the former than by the assumptions of conventional taxonomy.

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References and Notes

1. C. F. Culberson, *Chemical and Botanical Guide to Lichen Products* (Univ. of North Carolina Press, Chapel Hill, 1969), pp. 73–226.
2. W. L. Culberson, *Taxon* **18**, 152 (1969); *Annu. Rev. Ecol. Syst.* **1**, 153 (1970).
3. M. E. Hale, Jr., *The Biology of Lichens* (Arnold, London, 1967), pp. 92–96, 144–146.
4. Of the herbarium specimens examined, 92 percent of the esorediate individuals were fertile; 99 percent of the sorediate individuals were sterile.
5. M. E. Hale, Jr., *Contrib. U.S. Nat. Herb.* **36**, 193 (1965).
6. C. F. Culberson and H. Kristinsson, *J. Chromatogr.* **46**, 85 (1970); C. F. Culberson, *ibid.* **72**, 113 (1972).
7. The essentially allopatric ranges of the chemical races of the sorediate-sterile morph in the eastern United States result from well-differentiated niches, for the organisms grow so widely spaced on tree trunks that competitive exclusion does not (at least now) seem to be a factor.
8. Although long-distance dispersal may be the simplest explanation of this widely disjunct distribution, it is tempting to relate it to the ancient connection of the New World and the Old World.
9. Four of the five known specimens of the latter race came from hardwoods. The ecology of the widely ranging stictic-constictic-norstictic race of the asexual morph is surely more complex than our analysis indicates. For example, this race, associated with conifers in the eastern United States, often grows on the specialized bark of the cork oak (*Quercus suber*) in the Mediterranean. The polyphyletic nature of the asexual morph is underscored by the fact that this and the sexual morph both also have poorly known races that produce alectoronic acid, an orcinol-type depsidone not closely related to the compounds referred to in this report. Organisms with this chemistry are relatively rare, restricted to seashore trees and maritime scrub in the southeastern states, and so ecologically remote from the races discussed above that we did not consider them in this study. But that a race with this very different and unsuspected chemistry evolved independently in both morphs seems far less probable than that the sexual morph of this chemistry gave rise to a sorediate asexual morph.
10. J. Poelt, *Bot. Notis.* **125**, 77 (1972).
11. These are the exact two-tail probabilities of 2 by 2 contingency tables. R. A. Fisher, *Statistical Methods for Research Workers* (Oliver Boyd, London, ed. 5, 1934), pp. 99–101.
12. We thank J. Antonovics for help with the statistical analysis. Supported in part by NSF grants GB-8359, GB-25346, and GB-31172.

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DNA Polymerase Activities of Human Milk

Abstract. *DNA polymerases have been partially purified from human milk. A DNA polymerase detected by phosphocellulose chromatography is similar to the enzymes of RNA tumor viruses in that a hybrid of polyriboadenylate and oligodeoxythymidylate is a better template than is DNA. However, this polymerase differed from that of the RNA tumor viruses in its chromatographic behavior. Three different methods of detecting "reverse transcriptase" activity failed to correlate with the donor's family history of cancer.*

The reported morphologic and biochemical properties of particles found in human milk appear similar to the properties of known RNA tumor viruses. (i) "Viruslike" particles of several types have been detected by electron microscopy (*1*). (ii) RNA-directed DNA polymerase activity has been detected by two methods: ribonuclease-sensitive incorporation of radioactive thymidine triphosphate (dTTP) into trichloroacetic acid–precipitable material (*2*); and incorporation of dTTP into molecules with sedimentation coefficients of 60 to 70*S* (*3, 4*). The latter method simultaneously detects the presence of a high molecular weight RNA of a size characteristic of the RNA of RNA tumor viruses and of an enzyme synthesizing DNA that cosediments with this RNA. (iii) RNA extracted from human breast cancers has been found to hybridize specifically with DNA produced in vitro from the mouse mammary tumor virus (*5*), and DNA synthesized by a human milk sample has been reported to hybridize with the polysomal RNA of a human breast carcinoma (*6*). Thus, DNA polymerases and nucleic acids of hu-