Proc. Nat. Acad. Sci. USA
Vol. 70, No. 4, pp. 1186–1190, April 1973

Self-Fertilization and Genetic Population Structure in a Colonizing Land Snail

(allozymic variation/genic heterozygosity/inbreeding/monogenic strains)

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Communicated by Ernst Mayr, February 20, 1973

ABSTRACT **The pulmonate land snail *Rumina decollata* in its native Mediterranean range is a complex of monogenic or weakly polygenic strains generated by a breeding system of facultative self-fertilization. One strain colonized North America and now occupies much of the southern United States and northern Mexico. No genetic variation within or among populations in the United States was detected in an electrophoretic analysis of proteins encoded by 25 loci. These findings emphasize the potential for adaptive convergence in the genetic systems of hermaphroditic animals and plants.**

The influence of self-fertility on the amount and organization of genetic variation in natural populations has been explored extensively in plants (1), and to some degree in Protozoa (2), but has been relatively neglected in animals. Consequently, recent theory and empirical evidence relating to the population genetics of close inbreeding have developed largely from botanical research (3). Yet the occurrence of self-fertilization in snails and other hermaphroditic animals leads us to expect convergence between animals and plants in strategies of adaptation involving regulation of the genetic system through control of the breeding system.

We here report the discovery in the pulmonate land snail *Rumina decollata* of complete or near absence of within-population genetic variability associated with a breeding system of facultative self-fertilization. In its native Mediterranean range, the species exists as a series of monogenic or weakly polygenic strains. One strain colonized North America within historical times and now occupies the southern part of the continent without detectable genetic variation.

Rumina decollata, a member of the pulmonate family Achatinidae, was introduced to Charleston, South Carolina (and perhaps other points in eastern North America) before 1822 (4). By 1915 it had spread westward from South Carolina and northern Florida through Texas and southern Oklahoma (5) and had also colonized Mexico, Bermuda, and Cuba (4). When first reported in Arizona (Mesa) in 1952 (6) and in California (Riverside) in 1966 (7), it was well established and presumably had reached these areas many years before. In the southeastern United States, the species has a spotty distribution, occurring locally in some urban areas. It is much more common and regular in occurrence in Texas, however, where it inhabits gardens and agricultural areas and has also invaded riparian and other native habitats. By reason of its extensive range and regional abundance, *Rumina* ranks as one of the more successful colonizing snails in North America.

MATERIALS AND METHODS

Samples. North American localities and numbers of specimens of *Rumina* electrophoresed are as follows: *California:* Riverside, 58. *Arizona:* Tucson, 15. *South Carolina:* Charleston, 4. *Texas*: Lubbock, 16; Forth Worth, 23; Ballinger, 30; Waco region (4 localities), 55; San Marcos, 15; New Braunfels (2 localities), 32; San Antonio, 6; Seguin, 5; Luling, 2; Martindale, 20; Mansfield Dam, 13; Lake Austin Lodges, 4; Austin region (14 localities), 456. The code designation for the North American strain is ATX.

Mediterranean localities, code designations, and numbers of specimens electrophoresed are as follows: *France:* Farm near Arles (RFAF), 45; Hillside near Arles (HAF), 3.2 km from RFAF, 33; Fons Outre-Gardon, near Nimes (FOGF), 7; Botanical Garden, University of Montpellier (UMF), 9. *Tunisia:* Hotel Megara, Gammarth (MGT), 45; Mediterranean Marine Sorting Center, Khereddine (MCKT), 7; Well number 1 near Mornag (M1T), 8; Well number 2 near Mornag (M2T), 8 km from M1T, 27; Kettana Oasis (KT), 5. Collections were made in November, 1972. Additional individuals from these localities are being used in breeding experiments.

Electrophoresis. Methods of tissue preparation, electrophoresis, and enzyme staining were similar to those of Selander *et al.* (8). The following 25 enzymes were assayed: phosphoglucose isomerase (EC 5.3.1.9), two malate dehydrogenases (EC 1.1.1.37), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), five phosphoglucomutases (EC 2.7.5.1), two aspartate aminotransferases (EC 2.6.1.1), two peptidases (leucylalanine and leucyltyrosine as substrates), two leucine aminopeptidases (EC 3.4.1.1), eight esterases, and two indophenol (tetrazolium) oxidases. Alleles at each of the 25 loci were numbered in order of decreasing anodal electrophoretic mobility of their corresponding allozymes, with a value of 100 being assigned to the most rapidly migrating allozyme.

Breeding Experiment. To study the breeding system of *Rumina*, snails were reared individually or in pairs from eggs in 100 × 20-mm plastic petri dishes containing moist filter paper, lettuce, carrot, oatmeal, and calcium carbonate powder. Eggs laid by the isolates and pairs, beginning when the snails were 10 weeks old, were incubated between moist pieces of paper towel in petri dishes. The incubation period was 30 days.

RESULTS

Because previous reports on allozymic variation in *Cepaea* (9) and *Partula* (10) and our own studies of the introduced European snails *Helix aspersa* and *Otala lactea* and native species of the genera *Mesodon* and *Rabdotus* have demonstrated that terrestrial snails are highly polymorphic (an average of about 18% of loci heterozygous per individual), we were surprised to discover that all 456 individuals in samples from 14 populations in the Austin, Texas, region were homozygous and allelically identical at all 25 structural gene loci assayed electrophoretically. An examination of 298 more individuals from 19 other localities in Texas, California, Arizona, and South Carolina similarly failed to demonstrate variation, and thus suggested that all populations in the United States represent a single monogenic strain. Although the possibility of variation at loci not assayed in our analysis cannot be excluded, we are at least certain that the level of variability in *Rumina* is exceptionally low. [A similar absence of allozymic variability has been found in small, isolated populations of fish (11) and lizards (12), presumably as a result of genetic drift, but all previous studies of animal species with large, continental distributions have demonstrated extensive variation (13).] If genic heterozygosity in the North American populations of *Rumina* were equivalent to that of "normally" variable species, we would expect to have recorded more than 3300 heterozygotes in our sample of 754 individuals.

Reasoning that close inbreeding is the most probable cause of an absence of genetic variability in *Rumina* populations, we performed an experiment to determine if cross-fertilization is necessary for reproduction. Eggs were obtained from individuals from Austin, and the reproductive performances of 105 individuals reared in isolation from the egg were compared with those of 65 pairs of individuals reared together from the egg. All but two of the isolates laid fertile eggs, and the average numbers of eggs laid and hatched by the isolates were at least equivalent to those of the individual pair members (Table 1). Because genetic markers were not available, we cannot rule out the possibility that reproduction in the isolates involved parthenogenesis rather than self-fertilization. This possibility is unlikely, however, because parthenogenesis is rare in molluscs (14), and only a special homozygosity-enforcing type (15) could account for the genetic character of *Rumina* populations. For the same reason, we could not determine if cross-fertilization was involved in the reproduction of the pairs in our experiment.

Although malacologists generally believe that most pulmonate snails are obligate outcrossers (14), self-fertilization has been demonstrated in many species, both aquatic and terrestrial (14, 16), and for several other species structural modifications in the reproductive system suggest that self-fertilization is the normal mode of reproduction (17). *Rumina* appears to be a normal functional hermaphrodite with the usual anatomical provisions for copulation and resultant outcrossing. The structure of the penis is normal, sperm are produced, and copulation occurs (18). However, a glandular sac that may be a specialized provision for self-fertilization occurs at the junction of the tubular seminal vesicle and the hermaphroditic duct (19). [A similar structure occurs in the achatinid genus *Glessula* (20), but is otherwise unknown in pulmonate land snails (21).] Experiments using genetic markers will be required to determine the frequency of cross-fertilization in *Rumina*, but the reproductive performance of the isolates suggests that self-fertilization is a regular, if not the predominant, breeding system.

Since heterozygosity decays at a rapid rate within self-fertilizing lines (families) (3), we can now account for its absence in North American populations. But the monogenic character of these populations is not similarly explained, for self-fertilization *per se* will not account for the absence of variation among lines. We must suppose that the uniformity of North American populations reflects selection for a uniquely adaptive monogenic genotype or that there was no variation in the inoculum originally introduced from Europe. These hypotheses are, of course, not mutually exclusive.

In a survey of *Rumina* in its native range, we sampled five local populations in Tunisia and four in southern France. Most populations have unique combinations of alleles, and seven of the samples are strictly monogenic (Table 2). Two populations are weakly variable at a single locus. Of 45 individuals from a farm near Arles, France (RFAF), 41 are homozygous for the $Pgm\text{-}4^{87}$ allele, but two are homozygous for $Pgm\text{-}4^{93}$ and two are heterozygous for these alleles. In a sample of seven individuals from a garden in Khereddine, Tunisia (MCKT), four individuals are homozygous for the $Est\text{-}9^{100}$ allele and three are homozygous for $Est\text{-}9^{95}$. The five Tunisian strains share alleles (Table 2) and morphological features (Table 3) that distinguish them from the four French strains, a feature of variation reflected in the taxonomists' designation of three geographic subspecies centering in North Africa, southern France, and Greece, respectively (22). Within each subspecies many "varieties" have been distinguished, some of which probably correspond to strains of the type we have identified.

DISCUSSION

Rumina decollata is a species (or possibly several taxonomic sibling species) in which the existing genetic variation apparently is distributed largely among closely inbreeding strains. The monogenic identity of populations in the United States suggests that they were derived from a single strain, the origin of which is more probably Europe than North Africa.

Although genetic variance among lines was detected in only two of the nine Mediterranean populations, we suspect that populations composed of two or more distinctive lines are not uncommon and that the genetic system of *Rumina* is

TABLE 1. *Mean production and hatching success of eggs from Rumina decollata reared in pairs or as isolates*

Item	Experimental group and number: Pairs (65)	Isolates (105)
Period of egg production measured (days)*	47	40
Daily egg production per individual	1.58	1.68
Number of eggs incubated per pair or isolate	54	41
Hatching success (%) in first month	65.9	67.1
Hatching success (%) in second month	74.5	77.8

* Period of egg production measured from the first day of laying for each pair or isolate.

TABLE 2. *Alleles at 16 variable loci in populations of Rumina decollata**

Enzyme locus	Population									
	North America	France				Tunisia				
	ATX	RFAF†	HAF	FOGF	UMF	MGT†	MCKT	M1T	M2T	KT
6-Pgd	95	95	83	83	83	100	100	100	100	100
Pgi	50	100	50	50	50	50	50	50	50	50
Pgm-1	97	97	97	97	97	97	97	97	97	100
Pgm-3	96	96	—	—	—	100	100	100	100	100
Pgm-4	92	87/93	100	100	100	92	92	92	92	92
Pgm-5	80	80	80	80	80	100	100	100	100	100
Pep-2	95	95	95	95	95	100	100	100	100	100
Lap-1	98	95	100	100	100	92	92	92	92	92
Lap-2	90	90	93	93	93	100	100	100	100	100
Ipo-2	80	100	80	80	80	50	50	50	—	50
Est-5	95	100	—	—	—	90	90	90	90	90
Est-6	90	95	100	100	100	—	—	—	—	—
Est-7	—	100	100	100	100	—	—	—	—	—
Est-8	—	—	—	—	—	95	95	95	95	100
Est-9	—	—	—	—	—	93	100/95	100	91	95
Est-10	95	100	95	95	95	100	100	100	100	100

* Nine other loci are invariable: *Mdh-1, Mdh-2, Got-1, Got-2, Pgm-2, Pep-1, Ipo-1, Est-2,* and *Est-3*. Dashes indicate that the enzyme was not identifiable on gels.

† See *Methods* for explanation of coded locations.

broadly similar to that of strongly self-fertilizing plants, such as the annual grass *Festuca microstachys* (23). Owing to the low vagility of *Rumina* and a high probability of populations being founded by one or a few individuals, it is likely that monogenic strains repeatedly arise from polygenic populations through colonization events involving a single monogenic line.

So little is known of the ecology and behavior of *Rumina* that we cannot understand why self-fertilization is advantageous for it, but not for some other terrestrial snails. Because it is likely that the genus arose in Africa (22), and that *Rumina decollata* (the only living species) evolved in the arid environment of North Africa, we can visualize an early history of occurrence in small, highly disjunct populations, with dispersal frequently involving the transfer of single eggs or juveniles and a resultant premium on ability to self-fertilize (24). However, there is no reason to believe that *Rumina* only recently colonized the more mesic region of southern Mediterranean Europe, where it is widespread and common, for it is recorded from the Tertiary Period (as *Rumina seringi*) in France (25).

Our findings are significant for genetic population biology

TABLE 3. *Morphological variation in populations of Rumina decollata*

Population	Body color	Foot color	Shell color	Mean adult shell width (mm)	Mean egg weight (mg)
North America					
ATX	Light gray	Olive gray	Light brown; sparse black mottling	10.9	12
France					
RFAF*	Light gray; black dorsal line	Light yellow	Similar to ATX	10.0	17
HAF	Black	Olive gray	Dark brown; dense black mottling; purple cast at mouth	11.7	13
FOGF	Black	Olive gray	Similar to HAF	11.3	15
UMF	Dark gray	Olive	Similar to HAF	11.4	13
Tunisia					
MGT*	Light brown	Yellow	Light yellowish brown; black mottling on larger whorls only	11.4	21
MCKT	Light brown	Yellow	Similar to ATX	11.2	18
M1T, M2T	Black	Yellow	Dark brown; dense black mottling	11.9	22
KT	Dark brown	Yellow	Similar to ATX	—	17

* See *Methods* for explanation of coded locations.

in several respects. First, the success of *Rumina* in North America demonstrates that species can achieve extensive distributions and high population numbers in the apparent absence of genetic variation. Second, a comparison of *Rumina decollata* and *Helix aspersa* in North America shows that drastically different genetic systems and adaptive strategies are used by successful colonizing species (26). In the apparent absence of genetic variability, the adaptation of North American *Rumina* populations to local microniches and regional patterns of variation in environmental factors presumably depends entirely on phenotypic flexibility and plasticity (27). In contrast, *Helix*, which apparently does not self-fertilize (14), has in its colonization of western North America maintained the full genic variability characteristic of populations in its native European range, with the potential for adaptive adjustment of allele frequencies in relation to geographically variable environmental factors (Selander and Kaufman, in preparation). Third, our findings, taken together with previous reports of various degrees of self-fertilization in snails, other invertebrates (28), and fish (29), suggest that hermaphroditic animals have evolved genetic systems and population structures like those of plants. The degree to which self-fertilization affects species structure and speciation in animals is unknown (30), but the prevalent notion that it is of little evolutionary importance is perhaps erroneous.

An association between sessility and hermaphroditism in both plants and animals was identified by Darwin (31), and many recent authors have emphasized the advantages of self-fertilization in colonization (24, 32). But the adaptive significance of the capacity for selfing often extends beyond reproductive "insurance," important as this may be. For example, several lines of evidence suggest that the regulation of amount of inbreeding in populations is an important means of adjusting genotypic and phenotypic distributions to those of environmental resources (3, 33). Comparative analyses of specialized genetic systems in plants and animals should contribute to an understanding of this and other aspects of ecogenetic adaptation.

We thank R. S. Ralin for assistance in the research and preparation of the manuscript, A. R. Mead for his interest in our work and information on snail biology, M. Bergoin for assistance in collecting samples in France, and D. A. Levin for critical review of the manuscript. Others who provided samples or information are D. S. Dundee, T. W. Fisher, W. H. Heard, L. Hubricht, P. McGee, A. Metcalf, H. D. Murray, R. L. Packard, W. L. Pratt, P. R. Ramsey, M. D. Robinson, and L. T. Ross. Supported by NIH Grant GM-15769 and NSF Grant GB-15664. Field work in Tunisia was made possible through funds from the Smithsonian Foreign Currency Program. D.W.K. was a Postdoctoral Fellow on NIH Training Grant 5T01-00337.

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