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Genetic distinctness of three widespread and morphologically variable species of *Drupella* (Gastropoda, Muricidae)

M. S. Johnson[1], **R. L. Cumming**[2,*]

[1] Department of Zoology, University of Western Australia, Nedlands, Western Australia 6009, Australia
[2] Department of Marine Biology, James Cook University of North Queensland, Townsville, Queensland 4811, Australia

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Abstract. Corallivorous gastropods of the genus *Drupella* have caused considerable damage to corals at widely separated reefs in the Indo-Pacific. Morphological variability of *Drupella* species within and between areas has caused taxonomic confusion. To clarify the relationships, we examined allozyme variation at 16 gene loci in samples from Western Australia, Queensland and Japan. Within sites, the species *D. cornus*, *D. rugosa* and *D. fragum* were distinguishable individually by each of 9 to 11 loci, with average genetic identities of about 0.25. The differences extended across sites, whereas the conspecific genetic identities over distances up to 6000 km were 0.86 to 1.00, supporting the view that there are three widespread species of *Drupella*. Nevertheless, there is much variation within species for allozymes, size, shape and colour.

Introduction

Members of the muricid genus *Drupella* are corallivorous gastropods, distributed widely on Indo-Pacific coral reefs. In recent years, destructive feeding aggregations involving three species have been observed in four widely separated locations: Western Australia (Stoddart 1989; Osborne 1992), Japan (Moyer et al. 1982), the Philippines (Moyer et al. 1982) and the Marshall Islands (Boucher 1986). However, a history of taxonomic confusion in this genus has led to disagreement about which species are actually involved in these destructive aggregations (Wilson 1992).

We found reference to 12 species of *Drupella* in the literature. Many are not now accepted as valid, but there have been disagreements about synonomies. Thus, while Cernohorsky (1969) listed four species from Fiji, and Fujioka (1982, 1984) distinguished five species from the Ryukyu Islands in southern Japan, Wilson (1992) recognised only two, with a possible third, unnamed species. Cernohorsky (1969) identified *D. fragum* as a male form of *D. cornus* and Fujioka (1984) equated *D. dealbata* with *D. fragum*. In contrast, Wilson (1992) equated *D. elata*, *D. eburnea* and *D. dealbata* with *D. cornus*, and *D. concatenata* and *D. fragum* with *D. rugosa*.

The excessive taxonomic splitting was probably caused by the considerable morphological variation in shells, and exacerbated by the thick calcareous algae that invariably cover adult shells, often concealing morphological characters.

The taxonomic confusion has complicated ecological research on *Drupella* in two different ways. First, it is difficult to integrate research from different geographic areas until it is certain whether the same or different species are found at different locations. Hence, existing information on the biology and ecology of *D. fragum* and *D. cornus* in Japan (Moyer et al. 1992; Fujioka and Yamazato 1983; Awakuni 1989) and *D. cornus* in Western Australia (Forde 1992; Hilliard and Chalmer 1992; Nardi 1992; Osborne 1992; Turner 1992, 1994; Holborn et al. 1994; Johnson et al. 1993; Black and Johnson 1994) cannot be compared with current research in Queensland (Ayling and Ayling 1992; Cumming 1992) or elsewhere without confidence in the taxonomic entities.

Second, amongst co-occurring snails it is not clear which morphological types represent reproductively isolated species and which represent variations within species or populations. Research at Lizard Island, Great Barrier Reef (Cumming 1992), has identified three morphological types that differ in size, shape and colour of the shell, and size and shape of the shell nodules. The distinction of these three groups is further complicated by a fourth group of intermediate individuals and by considerable size variation within a single group (Fig. 1). It has not been clear which morphologies represent reproductively isolated species and which represent variation within species. Distinguishing the species prior to ecological studies is important because they are likely to differ in their respective distributions, demographies and impacts on coral communities.

* *Present address:* The Swire Marine Laboratory, The University of Hong Kong, Cape d'Aguilar, Shek O, Hong Kong

Correspondence to: M. S. Johnson

The present genetic study was prompted by these problems in understanding their ecology. Using gel electrophoresis of enzymes, we compared specimens collected from Queensland, Western Australia and Japan with the aim of clarifying the taxonomic relationships within and between widely separated locations.

Materials and methods

Based on shell morphology (Table 1), specimens were provisionally identified as *D. cornus*, *D. rugosa* or *D. fragum*. This was based on Fujioka's (1982) descriptions of *D. fragum* and *D. cornus*, Wilson's (1992) descriptions of *D. cornus* and *D. rugosa*, published photographs and descriptions of *D. fragum* (Moyer et al. 1992), examination of specimens sent to one of us (RLC) from Japan and observations of egg masses of the three morphotypes at Lizard Island by RLC. Egg masses of *D. fragum* described by Awakuni (1989) and of *D. cornus* described by Turner (1992) corresponded with egg masses that RLC observed for animals of corresponding shell types on the Great Barrier Reef.

As noted by Moyer et al. (1982), however, the identification of *D. fragum* remains tentative until type specimens can be examined. To insure continuity with future taxonomic studies, we have deposited voucher specimens in the Australian Museum [C169228 = *D. cornus*, Lizard Island; C169229 = *D. rugosa*, Lizard Island; C169230 = *D. fragum* (labelled "3rd sp."), Lizard Island].

The Queensland samples were collected from front- and back-reef sites around Lizard Island on the Great Barrier Reef (14°40′ S, 145°30′ E). We analysed 33 *D. rugosa*, 33 *D. fragum* and 17 *D. cornus* from these collections. The *D. rugosa* included individuals with yellow, white and purple apertures, a trait that Wilson (1992) suggested may vary geographically. Two additional samples were collected at Lizard Island: 16 snails that had shell characters intermediate between *D. fragum* and *D. rugosa*, making identification difficult (Fig. 1 Q, R) and 12 snails with the appearance of *D. fragum*, but which were smaller than other *D. fragum* (approx. 15 mm versus 20–23 mm long), despite having the thickened shell at the aperture typical of adults (Fig. 1 G, H).

The Japanese samples were from a random collection at Shirigai Bay, Otsuki Town, Kochi Prefecture, Shikoku Island, Japan (32°46′ N, 132°42′ E). It consisted mostly of *D. fragum* but included a few specimens of *D. cornus* and *D. rugosa*. We were therefore able to examine three *D. cornus*, 12 *D. rugosa*, and 19 *D. fragum* from Japan.

The Western Australian samples were collected from two sites. Twenty-three *D. cornus* were taken from Jackson Island (28°53′ S, 114°00′ E) in the Houtman Abrolhos Islands and six *D. rugosa* from Bundegi Reef (21°51′ S, 114°11′ E) in the Ningaloo Reef Marine Park. *D. fragum* is not known to occur in Western Australia. In addition to allowing genetic comparisons of the different species within each geographic area, these samples provide for measurement of geographic variation within each species, over distances of 5200 to 6000 km.

The snails were frozen live in liquid nitrogen and held at −80 °C. Genetic variation of enzymes was examined by standard starch-gel electrophoresis. Enzymes were extracted by grinding 1 volume of foot muscle in 2 volumes of 0.2 M Tris-HCl buffer (pH 8), containing 0.25 M sucrose, 0.1% (v/v) mecraptoethanol and 0.02% (w/v) bromphenol blue. Electrophoresis was carried out using the lithium hydroxide (LiOH; buffer 2), tris-EDTA-borate (TEB; buffer 6) and tris-maleate (TM; buffer 9) buffers of Selander et al. (1971).

Eleven enzymes, representing 16 gene loci, were examined: aspartate aminotransferase (E.C. 2.6.1.1; TEB buffer; *AAT* locus); glucose-6-phosphate isomerase (E.C. 5.3.1.9; TM buffer; *GPI1* and *GPI2* loci); isocitrate dehydrogenase (E.C. 1.1.1.42; TM buffer; *IDH1* and *IDH2* loci); malate dehydrogenase (E.C. 1.1.1.37; TM buffer; *MDH1*, *MDH2*, and *MDH3* loci); malic enzyme (E.C. 1.1.1.38; TEB buffer; *ME1* and *ME2* loci); mannose-6-phosphate isomerase (E.C. 5.3.1.8; LiOH buffer; *MPI* locus); leucyl proline and valyleucine peptidases (E.C. 3.4.-.-; LiOH buffer; *PEPLP* and *PEPVL* loci); 6- phosphogluconate dehydrogenase (E.C. 1.1.1.43; TM buffer; *PGD* locus); phosphoglucomutase (E.C. 2.7.5.1; TM buffer; *PGM1* and *PGM2* loci); superoxide dismutase (E.C. 1.15.1.1; TEB buffer; *SOD* locus). Names and abbreviations of the enzymes and associated loci follow Shaklee et al. (1990) and differ slightly from those used in earlier studies of genetic variation in *D. cornus* (Holborn et al. 1994; Johnson et al. 1993). For enzymes encoded by more than one locus, the loci were numbered in order of decreasing electrophoretic mobility of their corresponding allozymes. Alleles at each locus were labelled numerically according to the mobility of their corresponding allozymes, proportionally to that of the most common allozyme in *D. cornus*, which was given a value of 100. Alleles with cathodally migrating allozymes are indicated by a minus sign.

Genotypic and allelic frequencies at each locus in each sample were estimated by direct count. Departures of observed proportions of heterozygotes from Hardy-Weinberg expectations were tested with a goodness-of-fit χ^2 test, excluding those cases in which the expected number of either heterozygotes or homozygotes was less than four. Genetic similarities across all 16 loci between populations were measured with Nei's (1978) unbiased genetic identity, which includes a correction for sample size. The matrix of genetic identities was summarized by a UPGMA phenogram. The extent of genetic subdivision within each species was measured by Wright's (1978) standardized variance in allelic frequencies, F_{ST}, which is the proportion of genetic variation that is due to differences among populations. F_{ST} was calculated by the method of Weir and Cockerham (1984), which corrects for sample sizes and departures from Hardy-Weinberg equilibrium. These corrections can result in small negative values of F_{ST}. All calculations were made using Biosys (Swofford and Selander 1981), version 1.7.

Table 1. Morphological characters used to distinguish among three species of *Drupella*. 'Adult' refers to shells with a thickened outer lip and apertural denticles. Sizes are approximate means for all specimens observed, although size is quite variable (Fujioka 1982; Moyer et al. 1982; this study)

Trait	*D. cornus*	*D. fragum*	*D. rugosa*
Adult length	Large, ≈ 33 mm	Small, ≈ 22 mm	Medium, ≈ 27 mm
Nodules	Large and conical; 4 spiral rows	Small, narrow, flat; Variable in size	Medium; 5 spiral rows
Aperture colour	White or yellow	White	White, mauve, purple, orange, or yellow
Body colour	Pale yellowish-green	Variable, between *D. cornus* and *D. rugosa*	Dark olive-green, mottled with pale green and white specks
Operculum colour	Dark brown	Variable, between *D. cornus* and *D. rugosa*	Yellow-brown

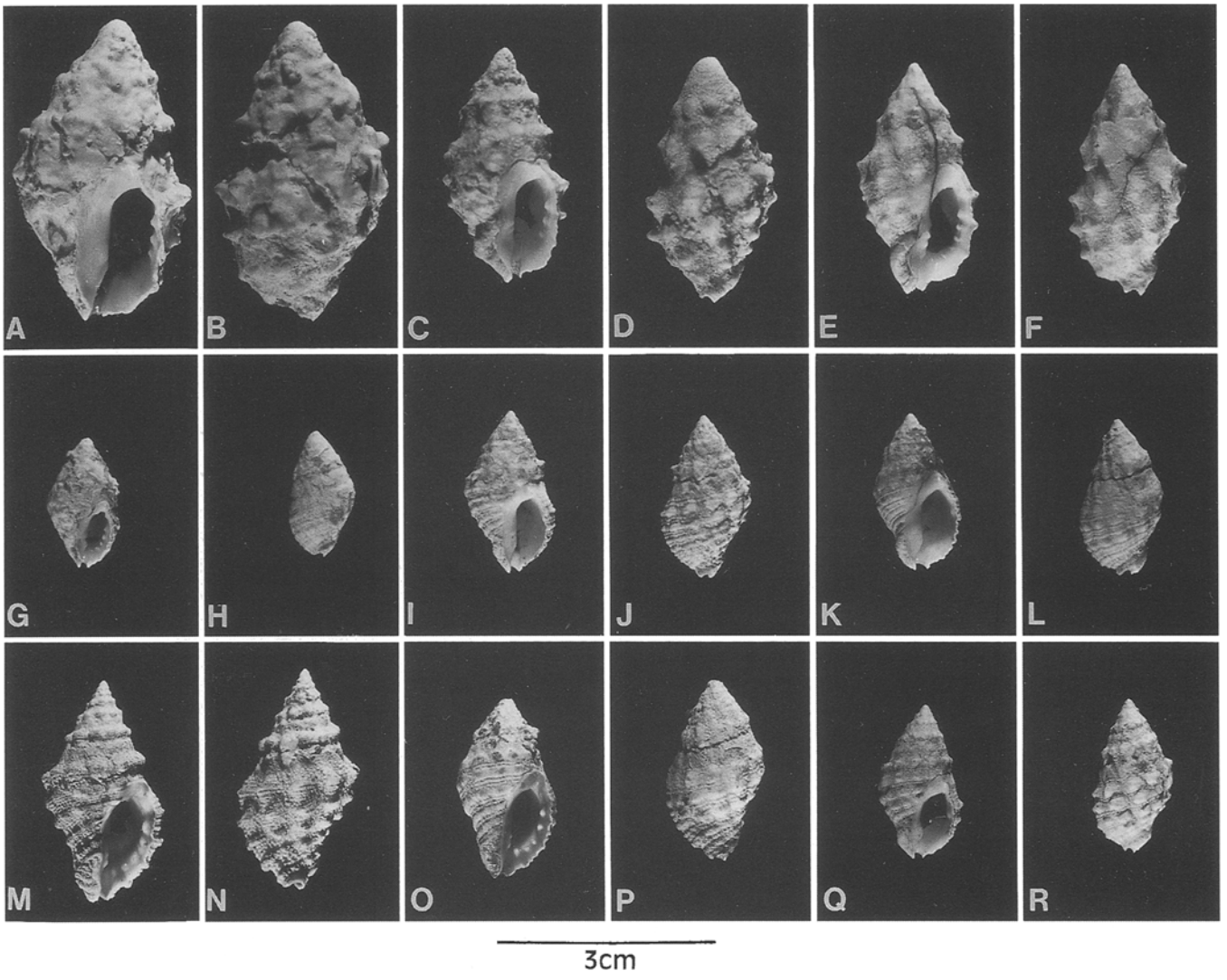

Fig. 1A–R. Examples of *Drupella* specimens analysed. **A, B**: *D. cornus* Western Australia; **C, D**: *D. cornus* Queensland; **E, F**: *D. cornus* Japan; **G, H**: 'dwarf' *D. fragum* Queensland; **I, J**: *D. fragum* Queensland; **K, L**: *D. fragum* Japan; **M, N**: *D. rugosa* Queensland; **O, P**: *D. rugosa* Japan; **Q, R**: *D. fragum* Queensland with shell characters intermediate between *D. rugosa* and *D. fragum* and analysed as possible hybrids

Results

All 16 loci varied within or between species and each species was highly polymorphic (Table 2). Multiple alleles were found at 13 loci in *D. cornus*, 9 in *D. rugosa* and 12 in *D. fragum*. Heterozygosity within populations averaged 0.244 and the estimates for the eight populations all fell within the range 0.208 to 0.292. Genotypic frequencies within populations conformed well with expectations from Hardy-Weinberg equilibrium. There were 48 valid χ^2 tests, of which only two were significant at $P < 0.05$ and none were significant at $P < 0.01$. This small number of apparent departures from Hardy-Weinberg equilibrium is no more than expected by chance, so there is no evidence against random mating within each species at each site.

Despite the high degree of variation within populations, the genetic differences between species were large and consistent across all samples examined (Table 1). Within areas, each pair of species could be distinguished (i.e. $\geqslant 95\%$ of individuals identified correctly) by each of 10 or 11 loci, at 8 to 10 of which the paired species had non-overlapping sets of alleles. The absence of heterozygotes for these diagnostic alleles confirmed the complete genetic distinctness of the co-occurring morphotypes. Examples of the analysed specimens are illustrated in Fig. 1. The morphological criteria used for provisional identification of specimens prior to analysis are summarized in Table 1. Our analysis shows that these criteria are reliable for distinguishing between species, and remain applicable over large distances. This scheme is also applicable to live specimens in the field. While the nomenclature remains provisional, there is no doubt that there are three morphologically distinguishable species.

The clear genetic groupings occurred despite considerable morphological variation within species. The 16 specimens from Lizard Island that appeared intermediate between *D. rugosa* and *D. fragum* were allozymically identical to the other *D. fragum*, confirming the absence of hybrids between these species. The 12 distinctly smaller snails from Lizard Island were also allozymically indistinguishable from the larger *D. fragum* (Fig. 1). Within *D. rugosa*, individuals at Lizard Island with white, purple or

Table 2. Allelic frequencies and observed heterozygosities (Het) at 16 loci in samples of three species of *Drupella* from Western Australia (W), Queensland (Q), and Japan (J). Sample sizes shown in parentheses

Locus	Allele	*D. cornus*			*D. rugosa*			*D. fragum*	
		W (23)	Q (17)	J (3)	W (6)	Q (33)	J (12)	Q (33)	J (19)
AAT	*25*	0.087	0.029	0.000	0.000	0.000	0.000	0.000	0.000
	−50	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	−100	0.913	0.941	0.667	1.000	1.000	1.000	0.000	0.000
	−200	0.000	0.029	0.333	0.000	0.000	0.000	0.000	0.000
	Het	0.174	0.118	0.667	0.000	0.000	0.000	0.000	0.000
GPI	*300*	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
	250	0.068	0.206	0.167	0.000	0.000	0.000	0.000	0.132
	225	0.000	0.000	0.000	0.000	0.106	0.292	0.000	0.000
	200	0.000	0.000	0.000	0.000	0.000	0.000	0.818	0.658
	150	0.000	0.000	0.000	0.000	0.394	0.208	0.000	0.000
	100	0.818	0.735	0.500	0.000	0.000	0.000	0.000	0.000
	75	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.211
	50	0.000	0.000	0.000	1.000	0.258	0.208	0.000	0.000
	25	0.114	0.029	0.333	0.000	0.000	0.000	0.030	0.000
	−25	0.000	0.000	0.000	0.000	0.242	0.292	0.000	0.000
	Het	0.261	0.235	0.667	0.000	0.697	1.000	0.242	0.474
IDH1	*120*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	0.000	0.000	0.000	0.032	0.026
	82	0.000	0.000	0.000	1.000	1.000	1.000	0.968	0.974
	Het	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.053
IDH2	*145*	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000
	120	0.283	0.250	0.167	0.000	0.000	0.091	0.000	0.000
	100	0.717	0.719	0.833	1.000	0.985	0.909	0.000	0.000
	75	0.000	0.000	0.000	0.000	0.015	0.000	0.985	0.974
	50	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.026
	Het	0.217	0.562	0.333	0.000	0.030	0.182	0.030	0.053
MDH1	*100*	0.978	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	85	0.022	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	Het	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH2	*126*	0.043	0.000	0.000	0.000	0.000	0.000	0.015	0.000
	110	0.000	0.000	0.000	0.000	0.000	0.042	0.439	0.474
	100	0.674	0.250	0.000	0.000	0.000	0.000	0.000	0.000
	85	0.000	0.000	0.000	0.000	0.000	0.000	0.545	0.526
	82	0.000	0.000	0.000	0.833	0.766	0.083	0.000	0.000
	72	0.283	0.750	1.000	0.000	0.000	0.000	0.000	0.000
	50	0.000	0.000	0.000	0.167	0.234	0.875	0.000	0.000
	Het	0.522	0.250	0.000	0.333	0.281	0.250	0.667	0.526
MDH3	*100*	0.870	0.647	0.667	0.000	0.000	0.000	0.000	0.000
	36	0.130	0.294	0.333	0.000	0.000	0.000	0.000	0.000
	28	0.000	0.000	0.000	0.000	0.000	0.000	0.984	0.842
	20	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000
	−10	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.158
	−18	0.000	0.000	0.000	0.000	0.094	0.000	0.000	0.000
	−36	0.000	0.059	0.000	0.000	0.000	0.000	0.000	0.000
	−52	0.000	0.000	0.000	1.000	0.672	1.000	0.000	0.000
	−124	0.000	0.000	0.000	0.000	0.172	0.000	0.000	0.000
	Het	0.261	0.471	0.667	0.000	0.458	0.000	0.031	0.316
ME1	*107*	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000
	100	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	93	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	Het	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ME2	*200*	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
	100	1.000	0.971	1.000	0.000	0.000	0.000	0.929	1.000
	25	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
	−10	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	Het	0.000	0.059	0.000	0.000	0.000	0.000	0.143	0.000

Table 2. (Continued)

Locus	Allele	*D. cornus*			*D. rugosa*			*D. fragum*	
		W (23)	Q (17)	J (3)	W (6)	Q (33)	J (12)	Q (33)	J (19)
MP1	*150*	0.043	0.000	0.000	0.083	0.000	0.000	0.000	0.000
	143	0.000	0.000	0.000	0.250	0.061	0.083	0.000	0.079
	100	0.326	0.794	0.667	0.583	0.273	0.125	0.136	0.184
	77	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026
	50	0.609	0.026	0.333	0.083	0.545	0.792	0.803	0.684
	7	0.022	0.000	0.000	0.000	0.121	0.000	0.061	0.026
	Het	0.615	0.412	0.667	0.500	0.606	0.333	0.381	0.579
PEPLP	*115*	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000
	108	0.022	0.000	0.000	0.167	0.061	0.042	0.061	0.079
	100	0.935	0.941	1.000	0.833	0.909	0.875	0.924	0.842
	95	0.043	0.029	0.000	0.000	0.030	0.042	0.015	0.079
	90	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
	Het	0.130	0.118	0.000	0.333	0.182	0.250	0.152	0.316
PEPVL	*135*	0.043	0.029	0.167	0.500	0.424	0.583	0.000	0.000
	119	0.065	0.000	0.000	0.250	0.227	0.167	0.000	0.000
	106	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.079
	100	0.761	0.676	0.333	0.167	0.333	0.250	0.000	0.000
	91	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.237
	81	0.130	0.294	0.500	0.083	0.015	0.000	0.000	0.000
	58	0.000	0.000	0.000	0.000	0.000	0.000	0.600	0.684
	Het	0.391	0.412	1.000	0.667	0.636	0.500	0.600	0.316
PGM1	*118*	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000
	112	0.000	0.000	0.000	1.000	1.000	0.958	0.000	0.000
	106	0.045	0.147	0.000	0.000	0.000	0.042	0.000	0.000
	100	0.955	0.853	1.000	0.000	0.000	0.000	0.091	0.105
	94	0.000	0.000	0.000	0.000	0.000	0.000	0.879	0.895
	Het	0.087	0.176	0.000	0.000	0.000	0.083	0.182	0.211
PGM2	*150*	0.023	0.000	0.000	0.000	0.016	0.000	0.000	0.000
	127	0.114	0.000	0.000	0.000	0.000	0.042	0.000	0.000
	122	0.000	0.000	0.000	0.000	0.047	0.042	0.000	0.000
	120	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
	119	0.364	0.088	0.000	0.000	0.000	0.000	0.000	0.000
	117	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.026
	114	0.000	0.000	0.000	0.167	0.203	0.208	0.625	0.211
	100	0.409	0.618	1.000	0.000	0.000	0.000	0.196	0.579
	93	0.023	0.000	0.000	0.417	0.484	0.417	0.000	0.000
	88	0.000	0.059	0.000	0.000	0.031	0.000	0.000	0.000
	82	0.023	0.176	0.000	0.000	0.000	0.000	0.000	0.000
	68	0.045	0.059	0.000	0.250	0.172	0.208	0.000	0.000
	58	0.000	0.000	0.000	0.167	0.047	0.083	0.018	0.184
	Het	0.739	0.529	0.000	1.000	0.606	0.583	0.571	0.579
PGD	*900*	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000
	800	0.000	0.000	0.000	0.000	0.152	0.042	0.000	0.000
	500	0.196	0.100	1.000	0.167	0.394	0.458	0.000	0.000
	350	0.000	0.000	0.000	0.000	0.015	0.000	0.690	0.763
	100	0.500	0.900	0.000	0.000	0.000	0.000	0.000	0.000
	50	0.000	0.000	0.000	0.750	0.409	0.458	0.000	0.000
	−200	0.000	0.000	0.000	0.000	0.000	0.000	0.310	0.211
	−500	0.304	0.000	0.000	0.083	0.015	0.042	0.000	0.026
	Het	0.609	0.200	0.000	0.500	0.545	0.583	0.414	0.263
SOD	*130*	0.000	0.000	0.667	0.000	0.000	0.000	1.000	1.000
	115	0.000	0.000	0.000	1.000	1.000	1.000	0.000	0.000
	100	0.804	0.571	0.333	0.000	0.000	0.000	0.000	0.000
	70	0.196	0.429	0.000	0.000	0.000	0.000	0.000	0.000
	Het	0.304	0.429	0.667	0.000	0.000	0.000	0.000	0.000
Mean Het		0.272	0.248	0.292	0.208	0.251	0.235	0.217	0.230

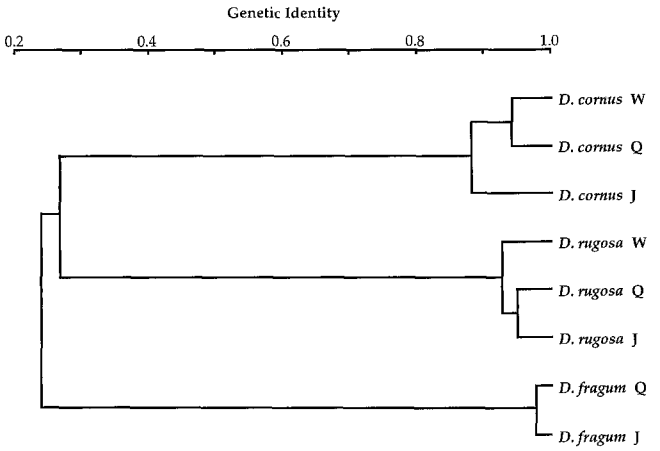

Fig. 2. Phenogram (UPGMA clustering) of genetic identities of samples of *Drupella cornus*, *D. rugosa*, and *D. fragum*. W, Western Australia; Q, Queensland; J, Japan

yellow apertures were electrophoretically indistinguishable.

The genetic differences between the species were consistent across the three geographic areas, as 10 or 11 loci consistently distinguished pairs of species regardless of location. The phenogram in Fig. 2 summarizes these results clearly, with three distinct clusters of conspecific populations. The genetic identities between species were very low, averaging about 0.25, whereas the genetic identities between conspecific populations were in the range 0.86 to 1.00. All pairs of the three species had similarly low genetic identities.

Although the conspecific groupings were clear, each of the species showed significant variation in allelic frequencies between these widely separated areas. For example, in *D. cornus* allelic frequencies at the *MDH2* and *MPI* loci showed two- to three-fold differences between the samples from Western Australia and Queensland, while the *PGD* and *SOD* loci had unique alleles at high frequencies in the samples from Western Australia and Japan respectively (Table 2). In *D. rugosa*, the largest differences in allelic frequencies were at the *MDH2* locus, for which the sample from Japan had a frequency of 0.875 for the *50* allele, in contrast to the frequencies of 0.167 in Western Australia and 0.234 in Queensland. Variation in *D. fragum* was less extreme, with only the *MDH3* locus showing even moderately large differences between the samples from Queensland and Japan. These intraspecific comparisons are summarized in terms of F_{ST} in Table 3. For *D. cornus* and *D. rugosa*, which were found in all three geographic areas, the average F_{ST} among all three samples was 0.161 and 0.128 respectively. For *D. fragum*, which was sampled only in Queensland and Japan, the average F_{ST} was smaller, at 0.054.

Table 3. Estimates of genetic subdivision, measured as F_{ST} in three species of *Drupella*

Locus	*D. cornus*	*D. rugosa*	*D. fragum*
AAT	0.090	–	–
GPI	0.025	0.196	0.036
IDH1	–	–	−0.021
IDH2	−0.042	0.047	−0.018
MDH1	−0.027	–	–
MDH2	0.335	0.428	−0.015
MDH3	0.057	0.133	0.130
ME1	–	–	–
ME2	−0.019	–	0.036
MP1	0.234	0.152	0.008
PEPLP	−0.031	−0.012	0.010
PEPVL	0.057	−0.014	−0.012
PGD	0.342	0.027	−0.006
PGM1	0.015	0.025	−0.020
PGM2	0.114	−0.032	0.223
SOD	0.215	–	–
Mean	0.161	0.128	0.054
± SD	0.045	0.066	0.045
Mean geographic distance (km)	5430	5030	5200

Discussion and conclusions

The most significant result of our genetic comparisons is that they confirm the existence of three genetically distinct species of *Drupella*. The coexistence of these species without interbreeding is clearest in our samples from Lizard Island, where we have examined moderate to large numbers of each species from the same reef. The absence of interbreeding between *D. rugosa* and *D. fragum* is especially interesting, because the presence of individuals with shells of intermediate characteristics had raised the possibility of some hybridisation, which can now be discounted. Genetic distinctness of all three species in sympatry is also clear from the Japanese samples, even though only three *D. cornus* were available for analysis. The samples of *D. cornus* and *D. rugosa* from Western Australia were collected from sites 400 km apart, so coexistence in sympatry is not as clear. However, an earlier study of allozymes in Western Australian populations of *D. cornus*, including Bundegi Reef, found very little geographic variation over distances up to 1170 km (Holborn et al. 1994), so we can conclude that *D. cornus* and *D. rugosa* are genetically distinct where they co-occur. The genetic differences among these species are consistent across the three widely separated areas examined. Although the names *D. cornus* and *D. rugosa* can safely be applied to two of these species, there are conflicting applications of the name *D. fragum* for the third species. Wilson (1992) considers *D. fragum* to be a synonym of *D. rugosa*, leaving the third species of *Drupella* without a name. Our results confirm that this relatively smooth-shelled species is the same entity in Queensland and Japan, but only careful examination of the type specimens will determine whether a new name is required. In the meantime, we continue advisedly to use the name *D. fragum* for this species.

The three species are not only genetically distinct, they are not even genetically close. The interspecific genetic identities of about 0.25 are very low, as some comparisons with other taxa make clear. In his summary of many

electrophoretic studies, Thorpe (1982) found that species with genetic identities this low had usually been placed in separate genera. On the other hand, similar comparisons between humans and chimpanzees showed genetic identities of about 0.5 (King and Wilson 1975), emphasising the discrepancies between morphological evolution and molecular divergence. Although notions of a molecular clock can be dangerous, the implications of the very low genetic identities amongst species of *Drupella* are worth noting. Based on the commonly used calibration of Maxson and Maxson (1979), the genetic distances suggest divergence times of about 23 million years amongst the three species of *Drupella*. Based on the much faster average rate of divergence found for the land snail genus *Partula* on Pacific Islands (Johnson et al. 1986), the times for divergence of species of *Drupella* are still greater than 10 million years.

The clarification of the genetic relationships within *Drupella* will facilitate the integration of studies in different places. It also raises questions about morphological variation within species; most noteworthy is the variation in *D. fragum* at Lizard Island. One stimulus for the present study was the suspicion of hybridization between *D. fragum* and *D. rugosa*, based on specimens with an intermediate shell appearance. The electrophoretic comparisons make clear, however, that these individuals are not hybrids, but *D. fragum*, emphasising the extensive morphological variation within a single population. That population also shows considerable size variation. One impetus for testing the relationships of a set of 'dwarf' *D. fragum* was Fujioka's (1984) description of *D. minuta* from Japan. *D. minuta* appeared similar to *D. fragum* (Fujioka 1984, plate 1) but was considerably smaller, similar to the small snails at Lizard Island. Our electrophoretic comparisons confirm that the 'dwarves' from Lizard Island are *D. fragum*.

In addition to the variation within populations, there is substantial geographic variation in size, shape and colour. While the electrophoretic comparisons confirm the underlying cohesion of each species, they also reveal substantial genetic subdivision over the large distances separating our sampling areas. Turner (1992) has confirmed that larval development is planktotrophic in *D. cornus* and a study of populations over 1170 km in Western Australia found little genetic subdivision ($F_{ST} = 0.007$), a result consistent with extensive gene flow (Holborn et al. 1994). At the larger geographical scale of the present study (mean distance of about 5200 km), however, the estimated values of F_{ST} of 0.161 and 0.128 for *D. cornus* and *D. rugosa* are relatively large for species with planktonic larvae. For example, Nishida and Lucas (1988) found F_{ST} to be 0.037 for the crown-of-thorns starfish over distances up to 7000 km in the western Pacific, and Winans (1980) found a value of 0.041 for milkfish over distances up to 9000 km. Over distances up to 3400 km across northern Australia, however, genetic subdivision of the pearl oyster *Pinctada maxima* is greater, with a mean F_{ST} of 0.104 (Johnson and Joll 1993). Although reduced gene flow across northern Australia relative to that over similar distances in the Pacific might contribute to the relatively high subdivision in *D. cornus* and *D. rugosa*, the two species do not show a consistent pattern of Western Australia being genetically more divergent. It will take more extensive sampling to determine whether the variation between the widely separated areas is geographically continuous or discontinuous and whether it shows similar or distinct patterns in the co-occurring species.

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