

MICROGEOGRAPHIC EVOLUTION OF SNAIL SHELL SHAPE AND PREDATOR BEHAVIOR

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Abstract.—Genetic divergence in geographically isolated populations is a prerequisite for allopatric speciation, one of the most common modes of speciation. In ecologically equivalent populations existing within a small, environmentally homogeneous area, an important role for environmentally neutral divergence is often found or inferred. We studied a species complex of conspicuously shaped *Opisthostoma* land snails on scattered limestone outcrops within a small area of lowland rainforest in Borneo. We used shell morphometrics, mitochondrial and nuclear DNA sequences, and marks of predation to study the factors involved in allopatric divergence. We found that a striking geographic divergence exists in shell morphology, which is partly associated with neutral genetic divergence. We also found geographic differentiation in the behavior of the snails' invertebrate predator and evidence of an evolutionary interaction between aspects of shell shape and predator behavior. Our study shows that adaptation to biotic aspects of the environment may play a more important role in allopatric speciation than previously suspected, even on a geographically very small scale.

Key words.—Adaptation, conchology, Gastropoda, Malaysia, Mollusca, *Opisthostoma*.

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Genetic divergence in geographically isolated populations is a prerequisite for allopatric speciation, generally considered one of the most common modes of speciation in animals (Barraclough and Vogler 2000; Gavrilets 2003; Coyne and Orr 2004). Such divergence may involve either adaptation to differences in the environment, or environmentally neutral divergence (e.g., by genetic stochasticity [Slatkin 1987] and cycles of Fisherian sexual selection [Iwasa and Pomiankowski 1995]), or a combination of processes. Evidence exists for environmental adaptation of populations in adjacent, distinctly heterogeneous habitat types (Endler 1980; Blondel et al. 1999; Schneider et al. 1999). However, when ecologically equivalent populations exist allopatrically within a small, environmentally homogeneous area, an important role for environmentally neutral divergence is often found or inferred (Mayr and Diamond 2001; Masta and Maddison 2002). Here, we use land snails to investigate adaptation to biotic components of apparently homogeneous habitat patches, as a factor in microgeographic divergence.

In view of their low mobility (Schilthuisen and Lombaerts 1994; Pfenninger et al. 1996) and their great intra- and interspecific shell variability, land snails are suitable organisms for studying geographic evolutionary differentiation (Davison 2002). Moreover, their empty shells provide a simple way for studying predation and selection in the field (Endler 1986; Vermeij 1987). We identified the Southeast Asian subgenus *Plectostoma* of the genus *Opisthostoma* (Caenogastropoda: Diplommatinidae) as a promising study system. These gonochoristic but sexually monomorphic (Schilthuisen et al. 2003a), microsnails (~2 mm tall) are characterized by shells in which the coiling direction appears to reverse in the last half whorl (Gittenberger 1995). They are common on limestone outcrops, but are completely absent from all other sub-

strates (Schilthuisen et al. 2003b). Conchological diversity is high: in Borneo, at least 50 species (defined morphologically only) are recognized, which differ greatly in shell shape and ornamentation (Vermeulen 1994). Almost all species are allopatric, and some that are endemic to single outcrops are endangered (Schilthuisen et al. 2005) and included in the IUCN Red List (IUCN 2004).

To investigate the evolutionary forces that may have shaped the shell divergence in this taxon, we studied the morphology, divergence in molecular markers, and predation in the *Opisthostoma concinnum* complex on 13 small (0.03–1.20 km²), isolated (mean separation 15 km) limestone outcrops along the lower Kinabatangan river in Sabah, Malaysian Borneo. These Miocene lenticular deposits have probably never been connected (Haile and Wong 1965) and are vegetationally very similar (Azmi 1998). Ten outcrops each harbor just a single population of the *O. concinnum* complex, whereas three (Gomantong, Tomanggong Besar, and Tandu Batu) each contain two distinct sympatric populations (Fig. 1a).

In our study, we first investigated whether genetic divergence has taken place in full allopatry. We then asked which aspects of the shell shape appear to evolve neutrally, and which may have evolved as a response to microgeographically varying predation.

MATERIALS AND METHODS

Field Procedures

We collected *Opisthostoma concinnum* sensu lato (in which complex we here also include the taxa *O. simplex*, *O. mirabile*, and *O. fraternum*) on the Lower Kinabatangan limestone outcrops between April 2001 and July 2005. The more distantly

related (Vermeulen 1994) outgroup species *O. jucundum* and *O. obliquedentatum* were collected at Mantanani Besar (116°20'55"E 6°42'42"N) in May 2002, and at Batu Sanaron (116°36'16"E 4°42'52"N) in February 2002, respectively. Live snails were preserved in 100% ethanol and stored at -25°C upon arrival in the laboratory. Empty shells were extracted from soil samples by flotation (Tweedie 1961). Voucher specimens have been deposited in the *BORNEENSIS* collection at Universiti Malaysia Sabah. (For full details of the 16 sampled populations, see Appendix 1 available online only at <http://dx.doi.org/10.1554/06-114.1.s1>.)

Morphometrics

For each population, 10 adult, undamaged snails were taken from the live-collected material and dried. Of each shell, digital photographs were taken at 50× magnification in apertural aspect (apertural plane perpendicular to the line of view), lateral aspect (apertural and umbilical planes parallel to the line of view), and umbilical aspect (umbilical plane perpendicular to the line of view). From these images, 17 linear measurements were taken (see Appendix 2 available online only at <http://dx.doi.org/10.1554/06-114.1.s2>), which were further analyzed in PC-ORD (McCune and Mefford 1999), unless otherwise specified. Each variable was first checked for normality in SPSS (2001). Only two characters, APEPOS and HEIAPE, were normally distributed. The others were normalized by log-transformation. To allow this, negative values were removed from CONSPI by adding 0.101 to all, and values of zero in all variables were changed to 0.001. Outlier analyses (based on a cutoff value of more than two standard deviations) revealed six outliers, which were removed to comply with the requirements for carrying out a principal component analysis (PCA). The data were then subjected to a PCA. The first three components extracted 66% of total variance, and 35% of all correlations were >0.30 and <0.75. A broken-stick eigenvalue analysis showed that only the first three components needed to be considered. Bartlett's test of sphericity in SPSS was significant ($P < 0.001$), and the Kayser-Meyer-Olkin measure of sampling adequacy was 0.78 (the raw measurements are given in Appendix 3, available online only at <http://dx.doi.org/10.1554/06-114.1.s3>).

Predation

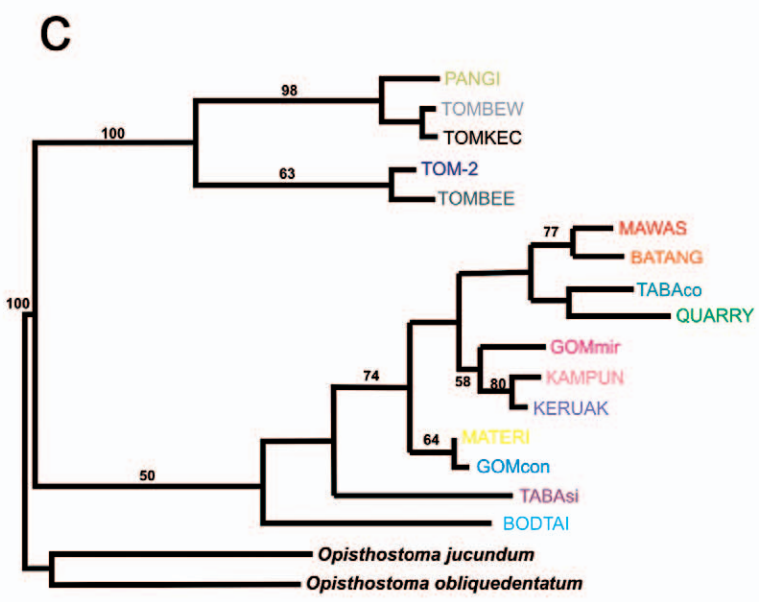
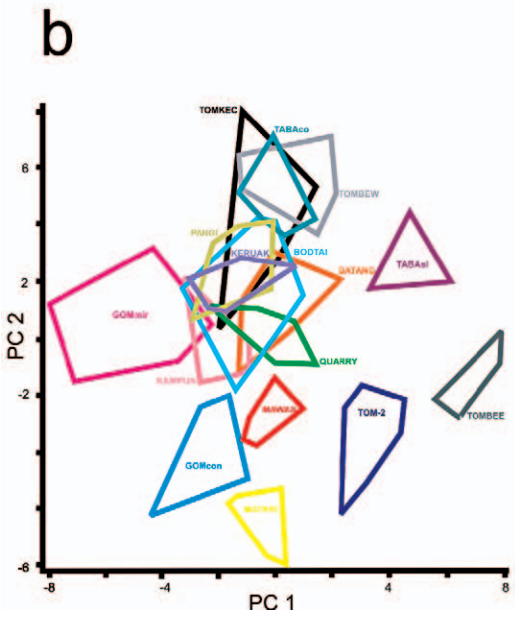
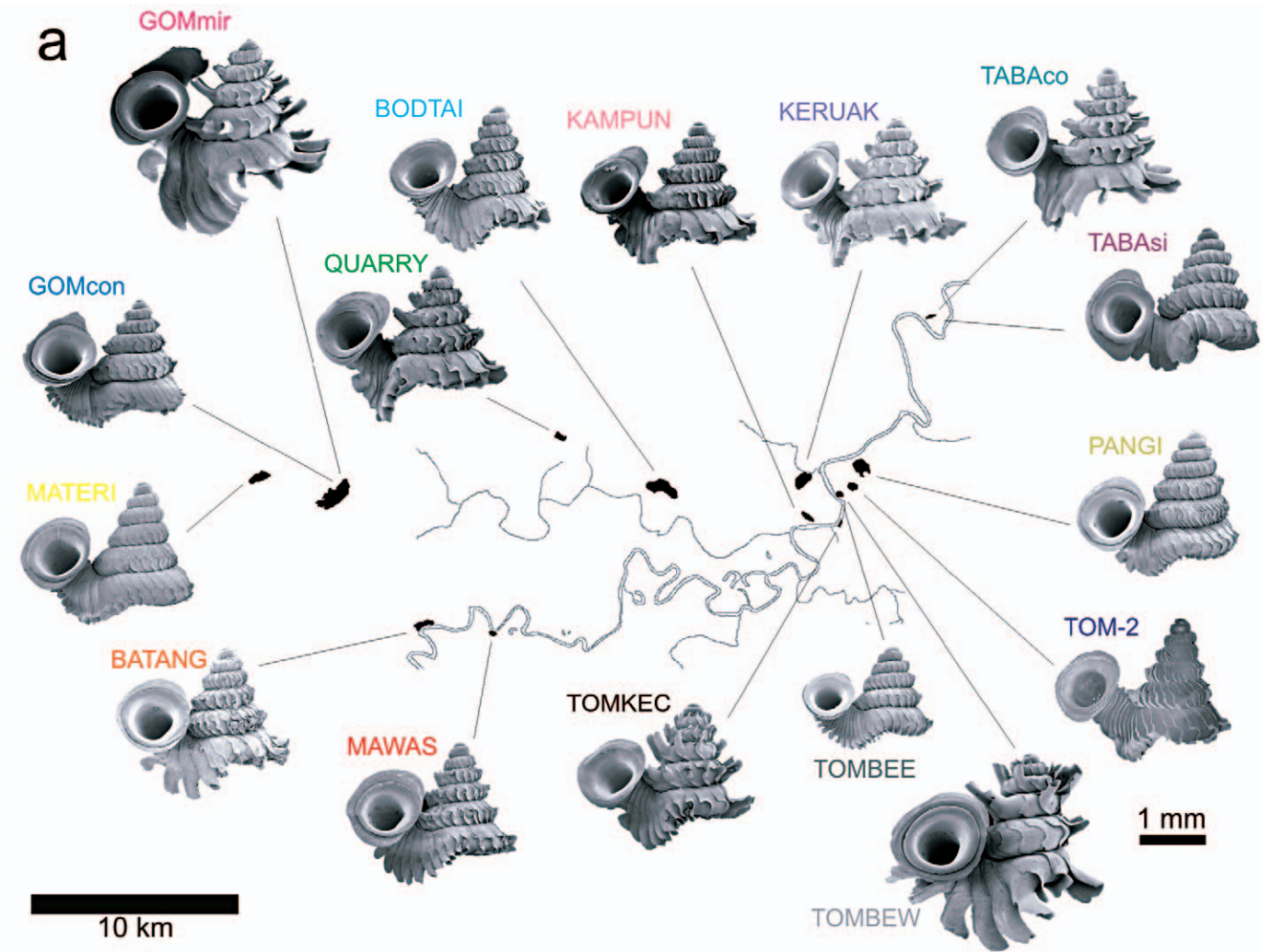
We observed the predatory behavior of an undescribed species, tentatively assigned to the slug genus *Atopos* (Gastropoda Pulmonata: Rathouissidae). Behavior was observed directly at Batu Tomanggong Besar at night (between 2000 and 2300 h), and indirectly by investigating the predation marks (bore holes) on empty shells from soil samples from all populations. All bore holes were very similar in size and shape, which suggests that they were all made by this predator. We divided the preferred positions of attack on the prey shell into 11 focal areas (A–K) and three additional areas (Fig. 2). For the nine populations with highest numbers of shells with bore holes (i.e., BODTAI, GOMmir, KAMPUN, KERUAK, TABAcO, TABAsi, TOMBEE, TOMBEW, and TOM-2), these 14 frequencies were used in a cluster analysis in PC-ORD, using Euclidean distances and group averaging. Counts of bore holes for each population are given in Table 1.

Molecular Phylogenetics

We extracted DNA from separate individuals using either a CTAB-based protocol (Winnepenninckx et al. 1993) or the Wizard genomic DNA extraction kit (Promega, Madison, WI). An approximately 400-bp region of the mitochondrial DNA (mtDNA) *16S* locus was amplified using the primer pair LR-J-12887 (Simon et al. 1994) and 5'ATTTAACGGCCGCAGTATCCT3'. We also amplified an approximately 700-bp fragment of nuclear rDNA (nrDNA), including the entire first internal transcribed spacer (ITS-1) and flanking portions of *5.8S* and *18S*, using the primer pair 5.8c "silkworm" and 18d "fruitfly" (Hillis and Dixon 1991). Polymerase chain reaction fragments were sequenced in both directions, either directly on an ABI 377 (*16S*), or after T-vector cloning on an ABI 3100 (*ITS-1*) (Applied Biosystems, Foster City, CA). We obtained *16S* sequences for two to 11 individuals per population, with the exception of GOMcon, in which amplification failed. For *ITS-1*, we obtained sequences for one to three individuals per population. The sequences have been deposited at GenBank under accession numbers DQ235707-235717 and DQ235719-235772. Sequences were initially aligned in ClustalW (Thompson et al. 1994) and then edited manually. We removed the invariant regions of the *18S* and *5.8S* genes and ambiguously aligned

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FIG. 1. The *Opisthostoma concinnum* complex consists of 16 populations. All are restricted to small limestone outcrops along the lower Kinabatangan River in Sabah, Malaysian Borneo. The populations have been identified with five- or six-letter codes: BATANG (*O. concinnum* at Batangan), BODTAI (*O. concinnum* at Bod Tai), GOMcon (*O. concinnum* at Gomantong), GOMmir (*O. mirabile* at Gomantong), KAMPUN (*O. concinnum* at Batu Kampung), KERUAK (*O. concinnum* at Keruak), MATERI (*O. concinnum* at Materis), MAWAS (*O. concinnum* at Mawas), PANGI (*O. concinnum* at Pangi), QUARRY (*O. concinnum* at Quarry), TABAcO (*O. concinnum* at Tandu Batu), TABAsi (*O. simplex* at Tandu Batu), TOMBEE (*O. concinnum* at the east side of Tomanggong Besar), TOMBEW (*O. fraternum* at the west side of Tomanggong Besar), TOM-2 (*O. concinnum* at Tomanggong 2), TOMKEC (*O. concinnum* at Tomanggong Kecil). All populations are allopatric, except those from Gomantong, Tandu Batu, and Tomanggong Besar. (a) Representative individuals for all populations; codes are explained in Appendix 1, available online only. (b) Principal components 1 and 2 for shell morphology. Most shell traits had loadings of >0.30 on only one axis: PC1: APEPOS, WIDAPE, HEIAPE, SPIHEL, SPIWID, SHEWID; PC2: SPARRS, MAXRRS, MAXRRT, SPARRT; PC3: MAXPET, MAXPEB, MAXPEL, DISOIP. (Of the remaining three traits, CONSPI and UMBWID had high loadings on more than one axis, whereas MAXPER did not have a high loading on any of the three major axes.) See Materials and Methods and Appendix 2 (available online only) for further explanations on the shell morphometrics. (c) An optimal tree of length 15,734 steps (base weight was 40 and gaps were weighted down according to their length), and a rescaled consistency index of 0.52. This tree was randomly selected from all 18 equally parsimonious trees. Only bootstrap values >50% are shown.



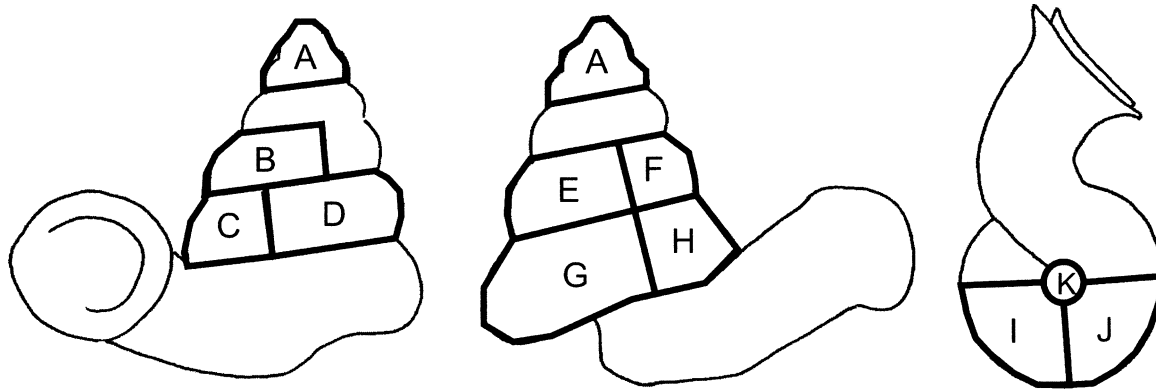


FIG. 2. The sectors on the shell that are preferred by *Atopos* as sites for bore holes. Sectors not marked with a letter were scored as: APERTURAL OTHER, remaining apertural sectors; ABAPERTURAL OTHER, remaining abapertural sectors; UMBILICAL OTHER, remaining umbilical sectors.

regions (total: 324 positions) from the *ITS-1* alignment, and weighted gaps by the inverse of their length (Giribet and Wheeler 1999). We then used PAUP*4.0 (Swofford 1998) for exploratory heuristic parsimony analyses on the *16S* and *ITS-1* datasets separately (maximum parsimony was preferred over other algorithms, because of the phylogenetically informative gaps; Giribet and Wheeler 1999). Except for sequences of TOMKEC and PANGI, which were almost identical, sequences from the same population always formed monophyletic groups with bootstrap values >50%. We chose a single sequence to represent each population. (We decided against using multiple or consensus sequences for the analysis, because consensus sequences do not necessarily exist in reality, for many populations only two sequences were available, and because for the *ITS-1* data, some regions in some sequences were poorly readable; thus, we chose to use the sequence with the highest quality.) A partition homogeneity test with 1000 replicates showed ($P = 0.09$) that both genes could be combined into a single data matrix with 126 parsimony-informative characters. This combined matrix was then subjected to a branch-and-bound search (with gap

weighting applied) and a bootstrap analysis with 500 replications. The tree of Figure 1c and the associated nexus-file have been deposited at Treebase (www.treebase.org) under accession number SN2515. A patristic distance matrix was then calculated (because of gaps and weighting, standard genetic distance measures could not be used).

Statistics

We used the Mantel test (9999 permutations) in PC-ORD (McCune and Mefford 1999) to investigate the correlations among matrices of geographic (see Appendix 4 available online only at <http://dx.doi.org/10.1554/06-114.1.s4>), patristic, predation, and PC1–PC3 centroid distances. For comparisons involving only within-clade patristic distances, and for bivariate correlations between shell characters and predation traits, we applied Pearson correlation in SPSS (2001) and two-tailed significance testing. Non-normally distributed variables were first log-transformed. Where appropriate, sequential Bonferroni correction (Rice 1989) was used. In the two pairs of sympatric populations, we used χ^2 to compare

TABLE 1. Predation frequencies overall and per shell sector for each population. Predation frequency (Freq.) was calculated from $N(p)$, the number of shells with predation marks, and $N(tot)$, the total number of shells. $N(s)$ indicates the number of shells for which the bore holes were scored per shell sector (for explanations of sectors A–K see Appendix 4, available online only). APO, apertural other; ABO, abapertural other; UO, umbilical other.

Population	$N(p)$	$N(tot)$	Freq.	$N(s)$	A	B	C	D	APO	E	F	G	H	ABO	I	J	K	UO
BATANG	11	53	0.20	11	2	0	0	0	1	0	0	3	1	0	2	0	0	2
BODTAI	44	178	0.24	44	0	4	1	7	7	5	3	3	3	1	7	2	1	0
GOMcon	14	151	0.09	14	0	0	0	1	5	2	0	1	3	0	0	0	2	0
GOMmir	36	367	0.09	36	18	2	0	3	5	0	0	1	0	0	4	0	3	0
KAMPUN	40	186	0.21	40	0	3	0	8	6	4	2	5	6	0	5	1	0	0
KERUAK	2789	13390	0.20	57	0	0	0	12	5	7	3	5	9	0	14	0	2	0
MATERI	9	208	0.04	9	0	0	0	1	1	0	0	1	0	0	1	2	3	0
MAWAS	3	203	0.01	3	0	0	0	0	1	0	0	1	0	0	0	0	0	1
PANGI	17	270	0.06	17	1	5	0	5	0	4	0	1	1	0	0	0	0	0
QUARRY	3	7	0.42	3	0	0	0	0	0	0	0	1	0	0	1	0	0	1
TABaco	60	302	0.19	60	0	0	12	4	2	2	0	2	1	0	7	7	23	0
TABasi	173	1050	0.16	95	0	1	20	2	4	0	1	0	1	0	4	0	62	0
TOMBEE	457	3204	0.14	94	1	9	2	28	6	5	15	6	14	3	2	1	2	0
TOMBEW	287	1495	0.19	133	2	6	11	26	6	20	19	16	13	4	9	0	1	0
TOMKEC	19	346	0.05	19	1	1	2	3	4	1	0	1	0	0	4	1	1	0
TOM-2	38	123	0.30	38	3	6	1	1	1	8	7	2	3	1	2	1	2	0

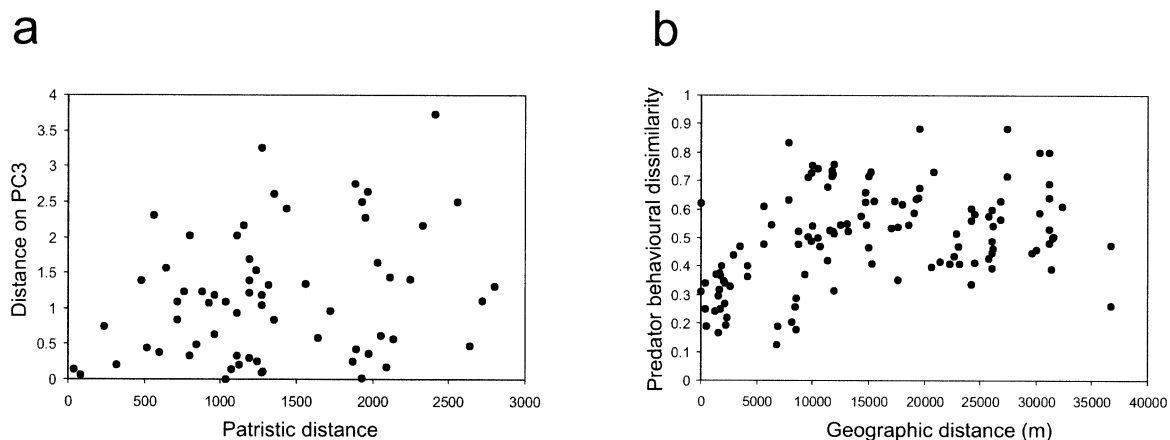


FIG. 3. Plots of (a) within-clade pairwise distances in PC3 (apertural ornamentation) and patristic (genetic) distance ($r = 0.411$, $P < 0.001$) and (b) pairwise dissimilarity in predator behavior and geographical distance ($r = 0.382$, $P = 0.003$).

the difference between each member of a pair in relative numbers of shells with bore holes. We also used χ^2 to compare the difference between each member of a pair in relative numbers of bore holes in each of the 11 focal areas A–K (see above).

RESULTS AND DISCUSSION

Morphological and Genetic Differentiation

To quantify morphological divergence, we measured 17 linear shell characters in 10 adult, undamaged individuals from each of all 16 populations. Principal component analysis was used to extract three major axes of variation. The loadings of characters on the principal components (PCs) reflect distinct groups of shell traits: general shell architecture (PC1), radial ribbing (PC2), and apertural ornamentation (PC3). Nine populations are fully separated along the three axes, whereas the remaining populations show partial or complete overlap (Fig. 1b). The fact that populations are morphologically quite homogeneous suggests that phenotypic plasticity may be limited.

To elucidate the phylogeny of this species complex, we sequenced an approximately 400-bp fragment of the mitochondrial *16S* gene, and the approximately 700-bp nuclear *ITS-1* from one to 11 individuals per population, and performed maximum parsimony analysis. This showed that all populations are monophyletic. We then phylogenetically analyzed both loci in one combined data matrix, using a single individual to represent its population. The resultant phylogenetic reconstruction (Fig. 1c) presents two clades: clade I composed of five populations in the southeast, and clade II containing the remaining populations. For *16S*, the mean pairwise Kimura's two-parameter genetic distances between the two clades is 0.081 (SD = 0.007). Ribosomal mtDNA evolutionary rate estimates are not yet available for terrestrial caenogastropods, and for other Gastropoda they range across two orders of magnitude (Reid et al. 1996; Chiba 1999). However, if we apply a median rate of 1% change per million years (Douris et al. 1998), this suggests that the group began diverging in the early Pleistocene. Genetic distances are not correlated with geographic distances for the entire group (r

= 0.03, $P = 0.303$) nor for within-clade comparisons ($r = -0.065$, $P = 0.304$). Moreover, in the three cases in which two populations occupy the same outcrop (GOMcon and GOMmir, both at Gomantong; TABAco and TABAsi, both at Tandu Batu; TOMBEE and TOMBWE, both at Tomang-gong Besar), these are never sister groups, making sympatric divergence unlikely. Together, these data indicate allopatric divergence under an island population structure due to incidental passive dispersal, similar to radiations in other insular habitats (Gillespie and Roderick 2002).

In such full allopatry, divergence may be due to genetic stochasticity, or driven by natural or sexual selection (Coyne and Orr 2004). In the *O. concinnum* complex, sexual dimorphism is absent, and the outcrops are geographically close and environmentally very similar (Azmi 1998). This would suggest that there is little opportunity for selection and that neutral divergence is the main cause for divergence. Indeed, significant linear correlations for within-clade patristic distance exist with apertural ornamentation (PC3; Fig. 3a) and shell architecture (PC1; $r = 0.272$, $P = 0.014$). However, these correlations disappear when comparisons between clades I and II are included, which indicates that an upper threshold to neutral divergence exists; moreover, radial ribbing (PC2) is correlated with geographic distance ($r = 0.301$; $P = 0.013$), but not with patristic distance ($r = -0.016$; $P = 0.451$). Although this may be due to a nonlinear correlation (and, hence, nonapplicability of Mantel testing), we will here pursue an alternative explanation, namely that factors other than neutral divergence also play a role in shell differentiation.

The Role of Predation

We discovered a geographically variable selection agent that can be implicated in adaptive shell divergence in this species complex. An undescribed, approximately 20 mm long predatory slug of the genus *Atopos* (Gastropoda: Pulmonata: Rathouissidae) inhabits crevices in the limestone and emerges at night to feed on *Opisthostoma*. The predator arrests its prey and uses its radula to scrape a hole in the shell wall through which its occupant is then extracted (Figs. 4b,c). We found that mean per-population predation frequency (calculated

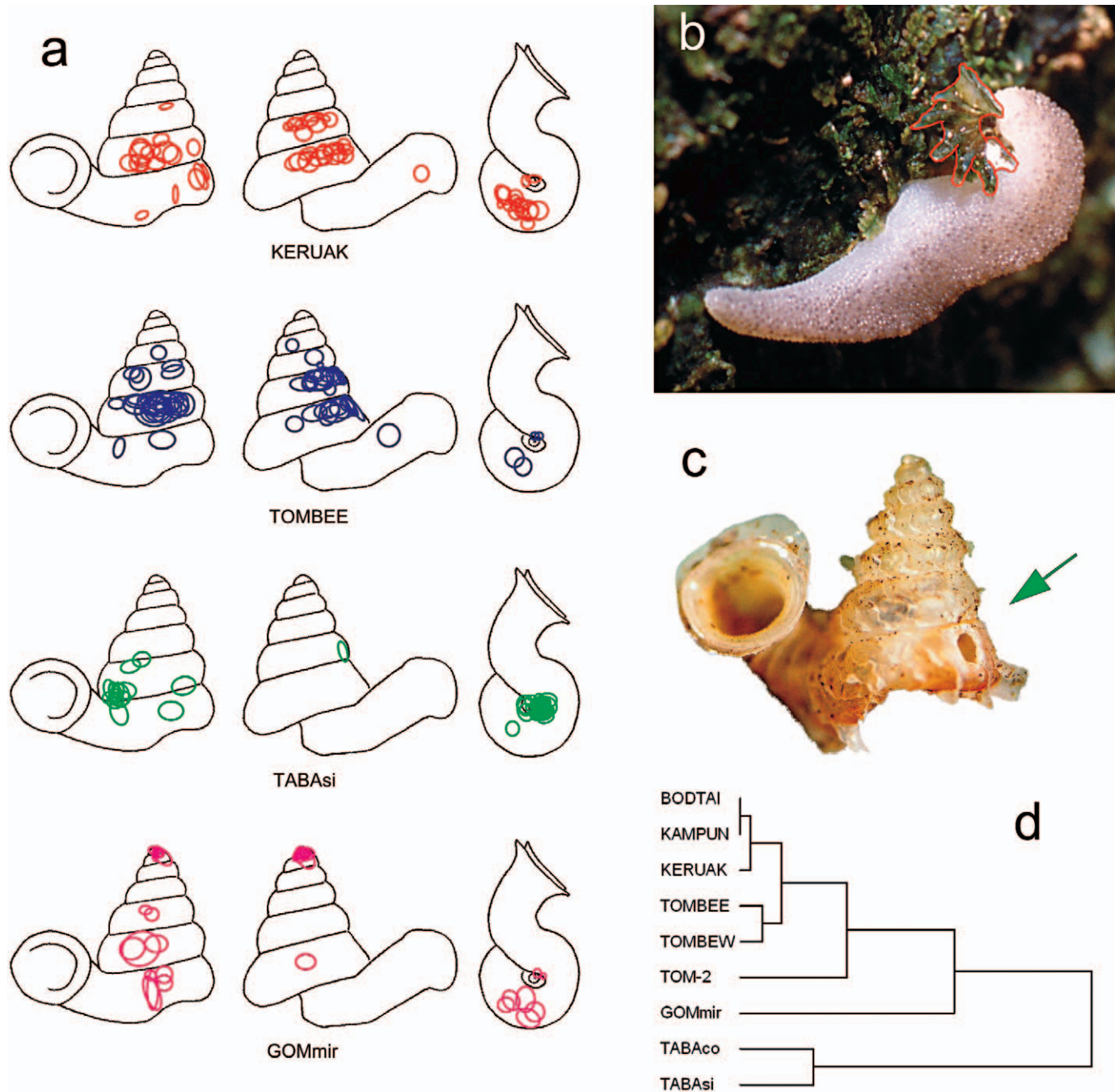


FIG. 4. *Atopos* predation in *Opisthostoma*. (a) Examples of geographical differences in the distribution of bore holes over the prey shell. (b) The rathouissid slug *Atopos* while boring a hole in an adult *O. concinnum* (outlined in red for clarity); photo by P. Koomen. (c) Appearance of a bore hole (arrow) in a shell of *O. concinnum* from Keruak. (d) Unweighted pair-group mathematic averaging (UPGMA) dendrogram showing the dissimilarity in distributions of bore holes in nine populations that share high predation frequency.

from bored shells divided by total shells) in adult *Opisthostoma* ranges from 0.01 to 0.31 ($n = 21,533$) and is correlated negatively with the height of the bottom part of the shell aperture ($r = -0.663$, $P = 0.005$). This shell character may thus be considered a general defensive trait that hinders one approach (via the concave part of the last half whorl) by which the predator can reach the shell wall with its mouth. More interestingly, *Atopos*' predatory behavior varies geo-

graphically (Fig. 4a): for all populations, we scored the positions on prey shells at which the predator gains entrance ($n = 663$). Using predation frequencies for 14 sectors of the shell surface, we find that the dissimilarity in predator behavior is significantly correlated with geographic distance (Fig. 3b). There may thus exist a selective pressure on *Opisthostoma* populations to evolve shell traits that obstruct the preferred manner of attack of the local *Atopos* population.

TABLE 2. Significant correlations between shell characters and character complexes on the one hand, and aspects of predator behavior on the other. Only correlations that remained significant after shell character-wide sequential Bonferroni correction (Rice 1989) are shown.

Shell variable	Predation sector	<i>r</i>	<i>P</i>	<i>n</i>
PC1	other abapertural	0.570	0.021	16
PC1	F	0.584	0.017	16
PC2	C	0.598	0.014	16
Aperture position (APEPOS)	C	-0.707	0.002	16
Umbilicus width (UMBWID)	C	-0.815	0.000	16
Width of spire (SPIWID) ¹	I ²	0.887	0.001	9
Shell width (SHEWID) ¹	I ²	0.843	0.004	9
Spire height (SPIHEI) ¹	I ²	0.854	0.003	9
Aperture width (WIDAPE) ¹	I ²	0.869	0.002	9
Aperture height (HEIAPE) ¹	I ²	0.881	0.002	9

¹ These shell traits are all correlated and form part of PC1.

² These comparisons were only significant when the seven samples with smallest samples size were excluded.

In this light, the situation on the outcrops Tandu Batu and Tomanggong Besar is instructive. On each of these, two conchologically distinct but sympatric populations are preyed on by the same population of *Atopos*. (The sympatric populations on the Gomantong outcrop are not considered, because predation frequencies are too low here.) We found that the predation patterns share outcrop-specific traits. On Tandu Batu, for example, both prey populations (which are syntopic) show high frequencies of attack in the shell umbilicus (38% and 65% of all bore holes for TABAco and TABAsi, respectively; Fig. 4a). This feature is almost absent from the populations on all other outcrops, where mean frequency of umbilical bore holes is only 5%. A dendrogram for predator behavior for the populations with highest predation frequencies, confirms the clustering of sympatric populations for these two localities (Fig. 4d). Thus, predator behavior follows local, probably genetically determined, stereotypes. At the same time, however, it is modulated by prey morphology, because predation frequencies, although similar, are significantly different (χ^2 -test; $P < 0.005$) for TOMBew (287/1495 = 19%) and TOMBEE (457/3204 = 14%), and predation patterns show significant differences for the two members of each pair of sympatric populations (Tandu Batu: $P < 0.005$; Tomanggong Besar: $P < 0.05$).

Both genetically stereotyped predator behavior and its modulation by prey shape are necessary conditions for coevolution between predator behavior and aspects of shell morphology. At Tandu Batu, for example, TABAsi, which has a frequency of bore holes in the umbilicus of 65%, also has a much narrower umbilicus (mean = 0.117 mm; SD = 0.019 mm) than TABAco (mean = 0.211 mm; SD = 0.032 mm), which suffers a frequency of umbilical bore holes of only 38%. Although these evolutionary interactions may only happen on a very small spatial scale, some are sufficiently general to cause certain shell characters to covary with aspects of predator behavior across all populations or subsets thereof. Most notably, PC2 (shell ribbing), which did not correlate with patristic distance, does show a correlation with predation at focal area C (Table 2). In addition, certain bivariate correlations between individual shell characters and aspects of predator behavior (i.e., preference for a certain focal area) are apparent (Table 2). It is perhaps not surprising that shell shape evolves in response to stereotyped predator

attack rather than, for example, shell thickness, because shell shape modification may be effected with relatively few costs in calcium requirements and growth rate.

We have shown that the members of the *O. concinnum* complex have diverged considerably both in shell shape and neutral genetic markers while in allopatry within a small geographic region. In at least three cases, divergence has been sufficient to allow secondary sympatry. Although some aspects of shell morphology undoubtedly diverge neutrally (and a yet unknown amount of phenotypic plasticity may be involved as well), others are impacted by the locally variable behavior of *Atopos* predators, and vice versa. We suggest that shell morphology, at least partly, may evolve in Red Queen (Van Valen 1973) cycles of predator-prey interactions in which the appearance of defensive structures in the prey is succeeded by evolutionary changes in predator behavior. The result is divergence in shell morphology. Although failure to culture *Opisthostoma* in captivity (Berry 1962; M. Schilthuizen, pers. obs.) has prevented experiments that might show a direct effect of shell morphology on reproductive isolation, we point out that courtship in these snails involves a stage in which one partner sits on the inverted last shell whorl of the partner (Schilthuizen et al. 2003a). It is thus not inconceivable that shell shape and ornamentation also play a role in mate recognition (Schilthuizen 2003).

Our study shows that allopatric speciation may involve unsuspected routes of adaptation, even in geographically very small and environmentally homogeneous areas. We suggest that detailed study of allopatrically differentiated populations may reveal that adaptation to ecological vagaries plays a more important role in allopatric speciation than previously recognized (Schilthuizen and Scott 2003).

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