Rapid evolution drives ecological dynamics in a predator–prey system

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Ecological and evolutionary dynamics can occur on similar timescales[1–7]. However, theoretical predictions of how rapid evolution can affect ecological dynamics[8] are inconclusive and often depend on untested model assumptions[8]. Here we report that rapid prey evolution in response to oscillating predator density affects predator–prey (rotifer–algal) cycles in laboratory microcosms. Our experiments tested explicit predictions from a model for our system that allows prey evolution[9]. We verified the predicted existence of an evolutionary tradeoff between algal competitive ability and defence against consumption, and examined its effects on cycle dynamics by manipulating the evolutionary potential of the prey population. Single-clone algal cultures (lacking genetic variability) produced short cycle periods and typical quarter-period phase lags between prey and predator densities, whereas multi-clonal (genetically variable) algal cultures produced long cycles with prey and predator densities nearly out of phase, exactly as predicted. These results confirm that prey evolution can substantially alter predator–prey dynamics, and therefore that attempts to understand population oscillations in nature[10,11] cannot neglect potential effects from ongoing rapid evolution.

In our experiments, parthenogenetic planktonic rotifers, *Brachionus calyciflorus*, consumed unicellular, obligately asexual[12] green algae, *Chlorella vulgaris*. These were cultured in continuous flow-through chemostat systems in a nitrogen-limited medium. A simple differential-equation model predicts the transitions between the qualitatively different population dynamics observed in this system (equilibria, population cycles and extinction) as a function of the nutrient concentration of the medium and the dilution (flow-through) rate of the chemostat[13]. However, cycle periods were far longer than predicted, and observed predator and prey cycles were almost exactly out of phase (predator maxima and prey minima were nearly simultaneous, and vice versa), which is impossible in a conventional predator–prey model[13]. These unexpected cycles had extended periods when algal biomass was high but rotifer densities remained low, followed by increased rotifer growth even though algal densities remained nearly constant.

To identify possible mechanisms that might account for these observations, we developed simple mathematical models for a series of alternative biologically plausible hypotheses[9]: changes in algal carbon:nitrogen ratio as a function of nutrient availability; rotifer self-limitation through toxin production or decreased egg viability when prey are scarce; and rapid prey evolution resulting from an evolutionary tradeoff between algal competitive ability and defence against rotifer predation. The model with rapid prey evolution reproduced the observed cycles much more accurately than our original model, whereas the non-evolutionary models failed to produce cycles similar to those observed[9].

Here we present experimental tests of the prediction that the qualitative properties of rotifer–algal cycles in our system are the result of rapid prey evolution. To test for the evolutionary tradeoff postulated by the prey evolution model, we manipulated algal growth conditions. Algae cultivated under constant and intense rotifer grazing pressure became lower in food value (in the sense that rotifers that fed on these cells had a lower population growth rate) and were heritably smaller and competitively inferior relative to algae grown in the absence of rotifers but with a comparable mortality rate imposed by an elevated chemostat dilution rate (Table 1). These results show the existence of different genotypes in the algal population, and the presence of a tradeoff between algal food value and competitive ability among clones. The heritable response to rotifer predation also shows that the selected low-food-value algal genotypes are better able to survive rotifer grazing, although the mechanism for this remains to be determined.

We added genetic structure, and therefore the possibility for algal evolution, to our original model[13] by representing the algal population as an assortment of clones differing in food value and competitive ability (Fig. 1). Clonal selection is appropriate because *C. vulgaris* reproduces asexually[12] and because we showed (above) the presence of different genotypes—clones—in our laboratory population (Table 1). The model incorporates the observed evolutionary tradeoff by assuming that lower-food-value clones have a higher half-saturation constant for nutrient uptake (Fig. 1a, c; see Methods for additional details).

A fundamental prediction of the multiple-clone model is that prey evolution can substantially alter the population cycles (Fig. 1). Model simulations for systems composed of randomly generated sets of one, two, three, five or seven clones show that when multiple clones are present, long cycles are possible (Fig. 1b, d) irrespective of the tradeoff curve specified between food value and competitive ability (Fig. 1a, c). Cycle lengths for two or more coexisting clones comprised a range of periods. Short cycles can occur when the algal population consists of two very similar clones, but with more than two clones short cycles become highly unlikely. In contrast, a single-clone system always produces relatively short cycles, regardless of that clone's phenotype. Moreover, population cycles in a single-clone system exhibit the classic predator–prey phase relations in which the peaks in predator abundance follow the prey peaks by one-quarter of a cycle (Fig. 1e). However, if the prey population consists of two or more phenotypically divergent clones, and predation by rotifers in combination with competition for nutrients results in rapid changes in algal genotype frequencies, the resulting multi-clone population cycles show phase relations like those observed, with algae and rotifers almost exactly out of phase (Fig. 1f). These properties result from the increasing dominance of low-food-value clones as the algal population is grazed down. Therefore, when algal density increases again after the rotifer population has crashed, the rotifer population does not immediately respond. Rotifers cannot increase until the algae return to high density, at which point higher-food-value clones (which are better competitors) increase in abundance. Rotifer grazing then intensifies and the cycle begins anew.

To test these predictions we manipulated prey evolution by altering clonal diversity (that is, genetic variability) in the prey population. We initiated replicated chemostat trials either with a single clone as an evolutionarily 'stagnant' population or with multiple clones comprising an evolutionarily 'active' one, at dilution rates and nutrient concentrations that had previously been shown to produce population cycles[13]. We then compared cycle periods and phase relations between the treatments.

Our results are strikingly consistent with theoretical predictions.

Table 1 **Evolutionary tradeoff for *C. vulgaris***

Treatment	Algal food value (d^{-1})	Competitive ability (d^{-1})
Rotifers present	1.07 ± 0.05	1.25 ± 0.02
Rotifers absent	1.58 ± 0.05	1.73 ± 0.03

Values are means ± s.e.m. Algal food value was measured by rotifer population growth rate when feeding on algae at sufficiently high concentration (see Methods). Competitive ability was estimated by algal growth rate under nutrient-deficient conditions (see Methods).

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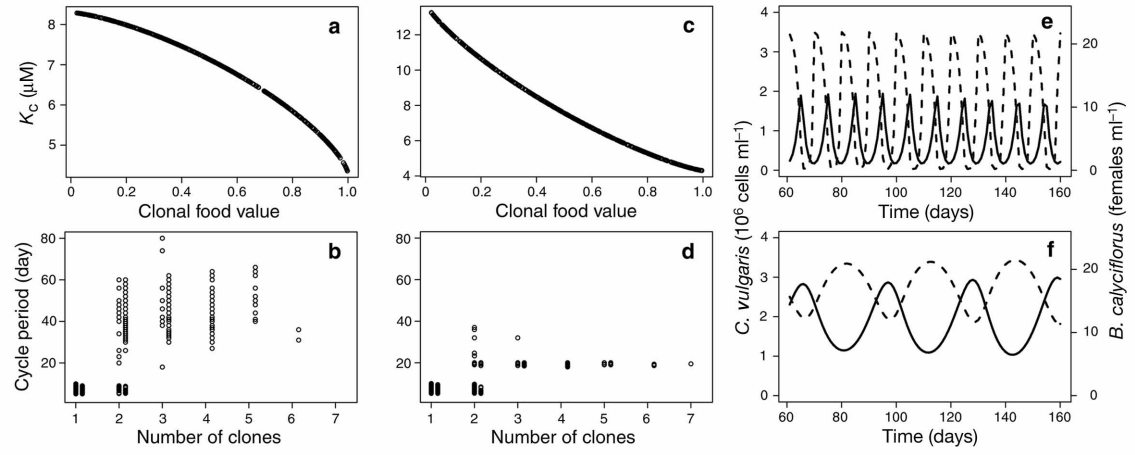

Figure 1 Predicted effects of prey clonal diversity on population dynamics. **a**, **c**, Modelled tradeoff curves between algal food value and competitive ability (represented by the half-saturation constant, K_c, for nutrient uptake by algae), with $\alpha' > 1$ (**a**) and $\alpha' < 1$ (**c**). **b**, **d**, Effects of clonal diversity on predator–prey cycle length for the two tradeoff curves between algal food value and competitive ability, with $\alpha' > 1$ (**b**) and $\alpha' < 1$ (**d**). Cycle periods in **b** and **d** are the result of 700 simulation runs each for one, two, three, five or seven clones present at the start of the run. Cycle periods are plotted against the number of clones remaining after running the model long enough for competitively inferior clones to be eliminated. 'Degenerate' cases (in which some of the initially present clones were eliminated) are plotted just to the right of non-degenerate cases in **b** and **d**. **e**, **f**, Cycles predicted by the model for the *Brachionus* predator (solid line) and *Chlorella* prey (dashed line) in a single-clone system (**e**) and a multiple-clone system (**f**). Model equations and further technical details are given in the Supplementary Information.

Population cycles of the single-clone evolutionarily stagnant treatment were much shorter and of smaller variance than those of the multiple-clone treatment (Figs 2 and 3). In addition, the single-clone treatments exhibited 'classic' predator–prey cycles with roughly a quarter-cycle delay between prey and predator maxima (Fig. 2; mean phase lag as a fraction of cycle period, 32%; range 26–36%). By contrast, in the multiple-clone evolutionarily active treatment, the predator and prey maxima were almost exactly out of phase (Fig. 2), as predicted by the clonal evolutionary model (mean phase lag, 56%; range 41–68%). The clonal evolutionary model is

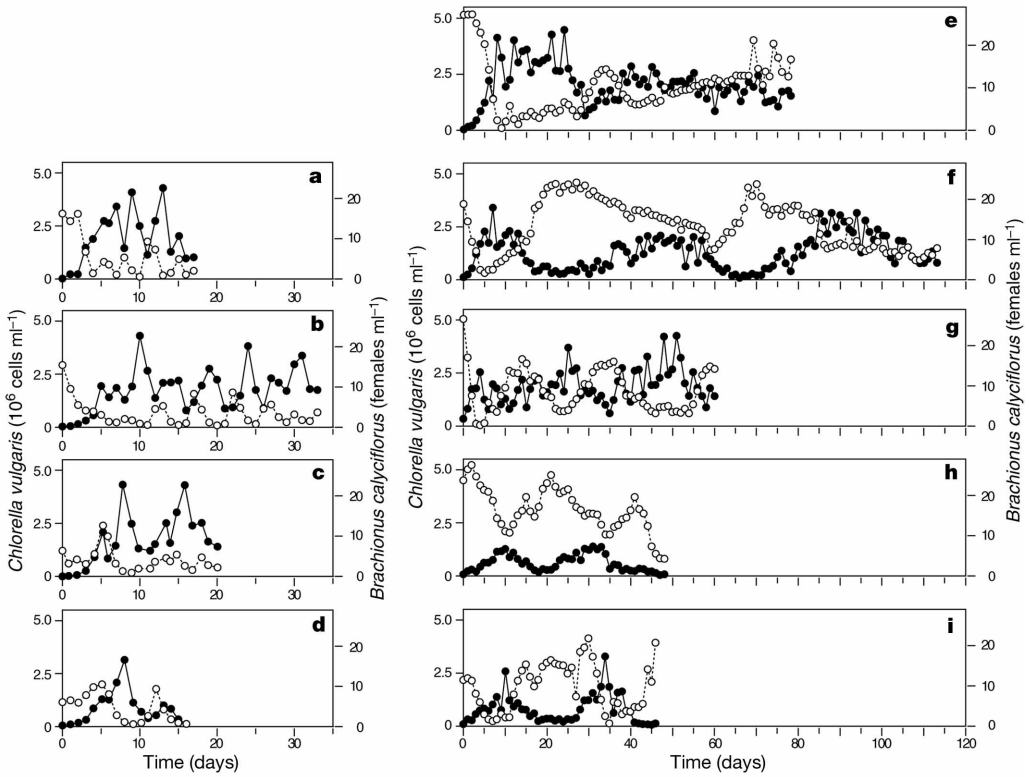

Figure 2 Experimental results showing the population cycles of rotifer–alga systems. **a**–**d**, Single-clone algal populations; **e**–**i**, multiple-clone algal populations. Filled circles, *B. calyciflorus* (predator); open circles, *C. vulgaris* (prey). In **a**–**d** there are short-period predator–prey cycles with the classical phase relations; in **e**–**i** there are long cycles with predator and prey oscillations nearly out of phase with each other. Dilution rates, δ (d^{-1}) **a**, 0.57; **b**, 0.65; **c**, 0.67; **d**, 0.68; **e**, 0.72; **f**, 0.64; **g**, 0.69; **h**, 0.95; **i**, 1.00. Data for four (**f**–**i**) of the five multiple-clone trials were obtained from ref. 13. The fifth trial is a new experiment, conducted in parallel with the single-clone trials, to verify that the lapse of time did not result in any change of our rotifer or algal stock cultures that would change the qualitative dynamics of a system with multiple algal clones. Note that population cycles at relatively high dilution rates (**h**, **i**) were also long and with out-of-phase relations, as our mathematical model predicts.

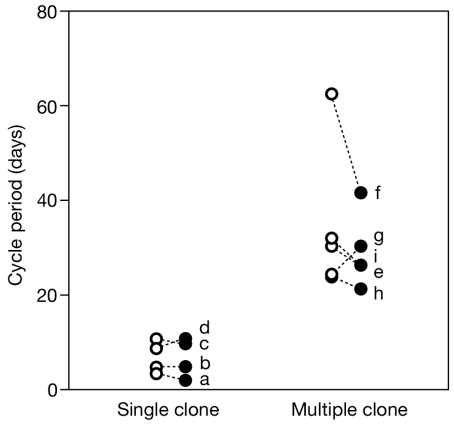

Figure 3 Cycle periods for experimental populations of *B. calyciflorus* (filled circles) and *C. vulgaris* (open circles). Lines connect period estimates from the same experimental trial, and the labelling of points corresponds to the panel labels in Fig. 2. The large difference between prey and predator period estimates by spectral analysis in experiment f resulted from the strongly non-sinusoidal shape of the cycles.

also able to match the experimentally determined bifurcation diagram of the system[13] (transition points between stable and cyclic dynamics as the dilution rate is varied) more accurately than the non-evolutionary model (see Supplementary Information).

Predators can also evolve rapidly[7]. Although we have not yet investigated predator evolution, it is significant that prey evolution alone brought our model into close agreement with experiments. Two explanations are possible. One is that the selection pressure of predators on prey might be stronger than that of prey on predators (the 'life–dinner' dichotomy[14,15]). Alternatively, predator evolution simply might not have much influence on our system's dynamics. Although inducible defences by prey are often observed in nature[16], none are evident in our system. Grazing-adapted cells are smaller but otherwise identical to non-adapted cells in morphology and chemical composition (C:N ratio; see Methods), and both types were predominantly unicellular. Inducible defences can also be ruled out as the cause of long-period cycles, because the cycle length and phase relations are both consequences of the fact that algal food value remains poor even after several algal generations without rotifer predation[9].

Our results provide experimental support for model-based approaches to identifying the causes of natural population cycles[11,17]. An ideal experimental test removes or alters a candidate causal process (as we have done here) and compares observed with predicted effects on dynamics. However, in natural systems such tests are typically infeasible because of the large spatial and temporal scales at which systems would have to be manipulated[18]. Even when such experiments are possible, their interpretation can be problematic[11]. An alternative is to construct mechanistic models corresponding to each potential causal mechanism and to compare each model's consistency with available observational and experimental data[11,17,19–21]. Our results here confirm the conclusion previously derived by this approach: that among all candidate causal processes only rapid prey evolution could account for our system's dynamic patterns.

We have shown that ongoing rapid prey evolution can alter population dynamics (cycle period and phase relations) in a live predator–prey system. Population cycles can lead to fluctuating selection, so that rapid prey evolution occurs indefinitely and reciprocally influences population dynamics. The interactions governing ecological dynamics are then continually changing through rapid evolution. Our study indicates that ecologists must consider ongoing rapid evolution when exploring the mechanisms underlying the dynamics of populations and food webs. Evolutionary and ecological dynamics must be understood in concert. □

Methods

Selection experiment

To assess variation in food value and competitive ability, *C. vulgaris* populations were exposed in two chemostats either to constant intense rotifer predation (dilution rate $\delta = 0.19\,d^{-1}$) or to unselective mortality from elevated washout ($\delta = 1.24\,d^{-1}$). Algal C:N ratios in the two treatments were similar (molar C:N = 13–14) and lower than in algae cultured under nutrient-deficient conditions ($\delta = 0.16\,d^{-1}$, molar C:N = 20), indicating that both treatments experienced an equivalent high availability of nutrients. Algae were harvested after each chemostat had reached equilibrium and then used to determine the effects of previous selection under common conditions.

Algal food value was determined by bioassay: rotifer population growth rate when fed with algae at a density (7×10^6 cells ml^{-1}) above the incipient limiting concentration[22]. Experimental procedures followed ref. 23. Algal competitive ability was estimated by measuring algal population growth rate under nutrient-deficient conditions in short-term batch cultures: 100 ml culture medium with 1 μM nitrate. Algae were inoculated at 500 cells ml^{-1}, grown at 25 °C under constant 120 $\mu E\,m^{-2}\,s^{-1}$ illumination (as in the chemostat experiments), and cell density and size distribution were monitored daily. Algal populations grew exponentially for the first 4–5 days; maximum growth rate was determined as the maximum derivative of a local polynomial curve fitted to the logarithm of algal density against time.

Simulation analysis

Model equations, parameter values and details are given in the Supplementary Information. Our model is based on that of ref. 13, a system of nonlinear differential equations for the state variables: limiting nutrient, algal abundance, breeding and total rotifer abundances. The two rotifer variables represent rotifer age structure, with rotifers gradually ceasing to breed with age; in other respects it is a standard chemostat model with Monod equations[24] for algal nutrient uptake, and for predation by rotifers on algae, with fecundity proportional to consumption. Here, we replaced the single algal variable by a series of algal clones. Clones are characterized by their relative food value to rotifers, p, ranging between 1 ('good') and ~0 ('poor'), with lower p resulting in reduced predation risk. The defensive 'low-food-value' trait comes at the expense of reduced competitive ability (Table 1). To model this tradeoff, lower-p clones are given a higher half-saturation constant for nutrient uptake and are therefore poorer competitors when nutrients are scarce (our previous model[9] made different assumptions that are inconsistent with subsequent experimental results (Table 1, and T.Y., unpublished data) and used quantitative trait dynamics rather than clonal selection).

We initialized simulations with $q = 1$, 2, 3, 5 or 7 clones with random p values (uniformly distributed on [0.02, 1]) and competitive ability assigned according to one of two different tradeoff curves (Fig. 1a, c). Competitive ability (represented by the half-saturation constant K_c) is related to food value by $K_c = K + \alpha(1 - p^{\alpha'})^{1/\alpha'}$, where K is the minimum value applying when $p = 1$, α is a scale parameter describing the cost of decreased food value and α' is a shape parameter determining the concavity of the tradeoff. Estimation of tradeoff curves is described in Supplementary Table 1. Clone sets were randomly drawn 700 times for each q. Clones comprising less than 1% of the final algal population were considered extinct. We then recorded the number of surviving clones and the cycle period (scored as period = 0 for coexistence at equilibrium).

Chemostat experiments

We established stock cultures of *Chlorella vulgaris* (UTEX no. 26) and *Brachionus calyciflorus* (taken originally from Milwaukee harbour, Wisconsin, and provided by M. Boraas). Although we do not know how much clonal (genotypic) variation for food value and competitive ability was present in our algal stock culture, populations derived from it in our selection experiment have been shown to possess distinct heritable phenotypes (T.Y., unpublished data), so we conclude that it consists of multiple clones. Isolated single algal clones were established by spot-plating cells from the stock culture on agar plates. We chose one of these arbitrarily for use in the experiments reported here.

The experimental chemostats followed ref. 13. To prevent algal contamination in the treatments using single algal clones, rotifers were taken from stock culture, rinsed thoroughly with sterilized medium, and held in sterilized medium for at least 6 h to ensure that all algal cells in their stomachs were digested. Rotifers were then thoroughly rinsed again with sterilized medium before being used to initiate chemostat trials. We set the dilution rate (0.57–1.00 d^{-1}) and nutrient concentration (80 μM nitrate) to give population cycles (ref. 13 and our model). Organisms were sampled daily through ports near the bottom and top of each chemostat. Rotifers were counted under a dissecting microscope and algae were counted with a particle counter (CASY 1; Schärfe, Reutlingen, Germany). Organism abundance data are means of duplicate samples. We determined cycle periods by spectral analysis of organism counts after rotifer populations were established (more than one female ml^{-1}) using spec.pgram in the R language[25], and phase lags from the cross-correlation function between predator and prey counts (ccf in R).

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Complex hybrid origin of genetic caste determination in harvester ants

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Caste differentiation and division of labour are the hallmarks of insect societies[1] and at the root of their ecological success[2]. Kin selection predicts that caste determination should result from environmentally induced differences in gene expression[3,4], a prediction largely supported by empirical data[5]. However, two exceptional cases of genetically determined caste differentiation have recently been found in harvester ants[6–8]. Here we show that genetic caste determination evolved in these populations after complex hybridization events. We identified four distinct genetic lineages, each consisting of unique blends of the genomes of the parental species, presumably *Pogonomyrmex barbatus* and *P. rugosus*. Crosses between lineages H1 and H2 and between J1 and J2 give rise to workers, whereas queens develop from within-lineage matings. Although historical gene flow is evident, genetic exchange among lineages and between lineages and the parental species no longer occurs. This unusual system of caste determination seems to be evolutionarily stable.

Pogonomyrmex barbatus and *P. rugosus* are common harvester ant species whose ranges broadly overlap in southwestern North America[9]. In both species, a population within the overlap zone in southwestern New Mexico has been found to possess a system of genetic caste determination[6–8]. By contrast, caste determination in populations outside the overlap zone is non-genetic[7,8], as is typical in ants[10].

To gain insight into the origin of genetic caste determination and the relationship between its occurrences in the two species, we conducted a genetic study of the two adjacent sites (Hidalgo[6] and Junction[7]) in which genetic caste determination has been described, and also in allopatric populations of *P. rugosus* and *P. barbatus* (see Methods). The three classes of genetic markers (allozymes, microsatellites and mitochondrial sequence data) revealed that the two sites are each composed of a distinct pair of interbreeding lineages, H1 (red-male[6]) and H2 (black-male[6]) at Hidalgo, and J1 (lineage X (ref. 7)) and J2 (lineage 4 (ref. 7)) at Junction. Each lineage has a unique multilocus genotype (Table 1) and is strongly differentiated from all other lineages (Nei's *D*, range = 0.53–1.50). Each lineage also contains a diagnostic monophyletic set of mitochondrial haplotypes (Fig. 1), showing a lack of genetic exchange across lineages.

As in previous studies[6–8], there were marked differences in the genomic composition of queens and workers at both Hidalgo and Junction. Of the 42 winged (young) queens collected at Hidalgo, 40 contained either an H1–H1 or H2–H2 genome, the remaining two having H1–H2 genomes. All 40 workers had an H1–H2 genome. A similar pattern was uncovered at Junction. Of the 38 winged queens, 37 had a J1–J1 or J2–J2 genome while one queen and all 35 workers had J1–J2 genomes. The few inter-lineage winged queens produced seem to have low reproductive success; we found no colonies displaying genotypes consistent with an inter-lineage mother (queen) at these sites or at five other sites surveyed in the region (22–40 colonies sampled per site, S. Helms Cahan and L. Keller, unpublished observations). We also did not find a single individual with an H–J genome of any type (H1–J1, H1–J2, H2–J1 or H2–J2), indicating that crosses between lineages from the two sites either do not occur or fail to give rise to viable females.

In addition to being genetically isolated from one another, all four lineages are genetically distinct from the *P. rugosus* and *P. barbatus* populations with non-genetic caste determination. Genetic distances between each species and the four lineages were uniformly high (*P. rugosus*, 0.35–0.85; *P. barbatus*, 0.56–1.39), with diagnostic differences at one or more nuclear loci between the two species and each of the four lineages (see Supplementary Information). Haplotypes of all four lineages were also clearly differentiated from both *P. rugosus* and *P. barbatus* (Fig. 1). Thus, neither *P. barbatus* nor *P. rugosus* seems to be currently linked by gene flow with any of the four lineages.

Two general hypotheses have been proposed for the origin of a two-lineage genetic caste system. The first is that a heterozygosity-based caste locus evolved within species, resulting in the splitting of the ancestral gene pool into two diverging lineages[7]. The second is that the evolution of genetic caste determination is associated with interspecific hybridization[6,8]. Our results show that the presence of distinct lineages within populations resulted from hybridization, most probably between *P. rugosus* and *P. barbatus*. Across nuclear markers, the two interbreeding lineages at each site clustered with different parental species: H1 and J1 with *P. rugosus*, and H2 and J2 with *P. barbatus* (Fig. 2). Moreover, the *cox1* mitochondrial haplotypes of J1 and J2 grouped together are paraphyletic (Fig. 1): the J1 clade is most closely related to *P. rugosus*, whereas that of J2 is