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Newly discovered reproductive phenotypes of a marine copepod reveal the costs and advantages of resistance to a toxic dinoflagellate

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Abstract

We document for the first time toxin-resistant reproductive phenotypes of copepods and we describe a novel procedure to identify these phenotypes. Individual copepods of the species *Acartia hudsonica* were raised on two diets: a standard nontoxic diet and a diet containing the toxic dinoflagellate *Alexandrium fundyense*, both offered at nonlimiting concentrations. Resistant individuals were defined as those that survived on the toxic diet. We examined several life-history characters including survivorship, age at metamorphosis, age at maturity, fecundity, and fitness. During this study, we discovered five resistance-related reproductive phenotypes that appeared as discrete classes in a frequency distribution of fecundity. After grouping the data according to these phenotypes, we calculated the fitness of each phenotype on each diet. We also calculated the cost and advantage associated with resistance. On the standard diet, one phenotype had 46% lower fitness than the phenotype with the highest fitness, indicating that possessing resistance alleles can carry a substantial cost. A different phenotype showed maximum relative fitness on the toxic diet and reduced relative fitness on the standard diet. From these results, we argue that resistance is conferred by a simple genetic system showing heterozygote advantage and leading to a polymorphism for resistance. Such a polymorphism will prevent the fixation of resistance alleles in natural populations. It may also confound the interpretation of typical experiments that measure average population responses.

Some phytoplankton, including dinoflagellates of the genus *Alexandrium*, can cause paralytic shellfish poisoning (PSP) in humans and other organisms through the neurotoxins that they produce (White 1981; Reyero et al. 1999; Garcia et al. 2004). But not all organisms respond to the toxins in the same way. Within a species or population, those exhibiting less severe symptoms of toxin poisoning for a given dose can be considered to be resistant to the toxin (Walker et al. 2001). Marine organisms that are resistant to the toxins of *Alexandrium* sp. include clams (Bricelj et al. 2005) and copepods (Colin and Dam 2002*b*; Colin and Dam 2004). The latter represent an important group of resistant animals for several reasons. First, copepods graze directly on toxic algae. Therefore, they may control through grazing harmful blooms of *Alexandrium* sp. Second, resistant copepods may accumulate toxins and therefore exacerbate the bottom-up transfer of toxins to fish, whales, and other predators (Turner and Tester 1997). In addition, the neurotoxins of *Alexandrium* sp. have sometimes been considered to deter grazing by copepods (Shaw et al. 1997; Teegarden et al. 1999). The relations between copepods and toxic *Alexandrium* sp. and the role of grazing in the development of blooms will be clearer when we understand the nature of resistance.

Resistance by definition confers a fitness advantage in the presence of the toxin. In these circumstances, resistant individuals will outperform nonresistant types through relatively better survival, growth, or reproduction. Such improved fitness in a stressful environment is generally accompanied by fitness costs—reduced fitness in a benign environment (Walker et al. 2001). Because the environment may not always be stressful, the costs incurred by resistant types in the absence of toxin are important to understanding how resistance may evolve. For example, high costs of resistance may slow or prevent the spread of resistance alleles throughout a population, whereas low costs may lead to the rapid spread of resistance alleles.

Resistance in the case of *Acartia hudsonica* refers to the ability of copepods to survive and reproduce while ingesting the toxic alga *Alexandrium fundyense*. Such resistance has been demonstrated in the copepod *A. hudsonica* in several studies. First, geographically separated populations of the copepod that have been historically exposed to the toxic alga showed significantly better survival and reproduction in the presence of *Alexandrium* sp. than populations that were not historically exposed (Colin and Dam 2002*b*). In another study, a nonresistant population responded within two or three generations to the introduction of the toxic alga in a laboratory selection experiment, increasing mean ingestion and egg production up to fourfold (Colin and Dam 2004). It was further demonstrated that the mechanism of resistance was not behavioral (avoidance), but was instead physiological (Colin and Dam 2003). The costs associated with this physiological resistance, however, were not examined in those studies.

In this study, we examined the life-history traits of individual copepods to test the hypothesis that resistance to the PSP toxins of *A. fundyense* carries a cost in the absence of the toxic alga. We reared copepods individually rather than as pooled cohorts to identify resistant individuals, and

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we defined resistant individuals as those that survived in a toxic diet treatment. During the course of this study, we discovered discrete reproductive phenotypes related to resistance, and we quantified both the fitness advantage enjoyed by each phenotype as well as the cost of resistance to each phenotype. From these results, we argue that toxin resistance carries a cost, and that resistance may be conferred by a simple genetic mechanism showing heterozygote advantage.

Materials and methods

Copepod and algal cultures—The copepods used in this experiment came from two laboratory populations, a control line (never exposed to *Alexandrium* sp.) and a selected line (fed a diet containing *Alexandrium* sp. for many generations in the lab). Both lines had been kept in the laboratory for approximately 4 yr before the start of the experiment. They are the same lines reported in Colin and Dam (2004) and they originated from *A. hudsonica* individuals captured from Great Bay, New Jersey. Wild field-caught copepods (from Great Bay) were added to the control line (see below) once each year. Before this experiment (February 2003), the control line was last augmented with new copepods in May 2002.

The copepods were maintained in 20-liter pails of filtered seawater at 15°C and ambient day length under fluorescent lighting. One of the lines (control) was fed the standard diet (three times per week), a mixture of nontoxic algae (*Thalassiosira weissflogii, Isochrysis galbana*, and *Tetraselmis* sp.). The other line (selected) was fed a toxic diet that included the algae of the standard diet (three times per week) plus the toxic *A. fundyense* (isolate BF-5—equivalent to NB05 in Colin and Dam [2002*a*]) (once per week). The total concentration of phytoplankton in the water immediately after feeding was approximately 10^3 μg L^{-1} C. The concentration of *A. fundyense* cells in the pails immediately after feeding was approximately 10 cells mL^{-1}.

The phytoplankton cultures used to feed the copepods were grown in semicontinuous culture using F/2 growth medium. The 2-liter cultures were diluted with growth medium approximately once each week. They were kept at 18°C in an environmental chamber equipped with fluorescent lights set to a 12 : 12 light : dark (LD) photoperiod.

Experimental design—To reduce potential maternal effects from the selected line, eggs and nauplii from both lines were transferred from the mass rearing pails to separate nearby 4-liter beakers, fed the standard diet, and allowed to mature (F_1 generation). Food was provided three times per week and water changed once per week. When the copepods in the 4-liter beakers matured, approximately 50 gravid female copepods were placed in egg-catching devices (1 liter), fed the standard diet, and allowed to produce eggs over 24 h. After the eggs hatched (F_2 generation), 90 individual nauplii were collected from each of these cohorts and isolated in 60 × 15 mm petri dishes containing one of two food mixtures, marking the beginning of the experiment reported here. It is important to note that by this time the copepods from the selected line had been removed from the toxic diet for two generations.

Two mixtures of algae were used as food in the experiment. The control mixture consisted of *T. weissflogii* (~11 μm diameter), *I. galbana* (~4 μm), and *Tetraselmis* sp. (~7 μm) in equal proportions (by carbon) at a total of 600 μg L^{-1} C, a nonlimiting concentration (Colin and Dam 2003). The cell sizes and cell concentrations were measured with an Elzone 280 particle counter. The average carbon content of each algal clone was estimated from calculated cell volume and carbon–volume relations made in previous lab experiments (Colin and Dam 2002*b*). The toxic diet consisted of a combination of the nontoxic algae mentioned and the toxic *A. fundyense* (~25 μm). The toxic diet also totaled 600 μg L^{-1} C but 25% of it was from *A. fundyense*. The toxic cell concentration was approximately 40 cells mL^{-1}. The toxin content of the *A. fundyense* cells was determined by high-performance liquid chromatography (by D. Kulis, Woods Hole Oceanographic Institution) after extraction in 0.1 mol L^{-1} acetic acid (Anderson et al. 1990). Saxitoxin derivatives measured included saxitoxin, neosaxitoxin, gonyautoxins 1 and 3, and gonyautoxins 2 and 4. The toxin content of the BF-5 was measured at the beginning and at the end of the experiment. The average cellular toxin content was 11.3 pg of saxitoxin equivalents (SD = 0.94, n = 4). During the course of rearing the selected line of copepods, we periodically tested the *A. fundyense* cultures for toxicity. It generally ranged between 10 and 40 pg of saxitoxin equivalents per cell.

Copepod development was tracked by examining each petri dish under a dissecting microscope every day or two. Developmental stage, mortality, and egg production of adult females were recorded. The food medium in the petri dishes was refreshed every other day for juveniles or daily for adult copepods. The petri dishes containing copepods were kept in translucent plastic containers with lids. The plastic containers were kept on shelves in a walk-in environmental chamber at 15°C and at a 12 : 12 LD photoperiod with diffuse fluorescent lighting.

Upon maturation of the copepods, sex was noted. After female copepods matured, they were transferred to larger petri dishes (60 × 20 mm) and their egg totals were tracked approximately daily until the end of the experiment. Approximately once each day, each female copepod was moved to a new petri dish, and her eggs were counted under a dissecting microscope. Moving the females reduced cannibalism of eggs. We occasionally observed that eggs had been cannibalized. In this case we counted the empty eggshells and included them in the total. After the experiment, the prosome lengths of female copepods were measured at ×100 using a compound microscope fitted with an eyepiece reticle after calibration with a stage micrometer. The experiment was terminated when all the copepods in both cohorts had either died or matured. Only time to metamorphosis and time to maturity were recorded for males.

Initial data analysis—Life-history traits, including adult size (prosome length), time to maturity (development time or age at maturity), time to metamorphosis (time to

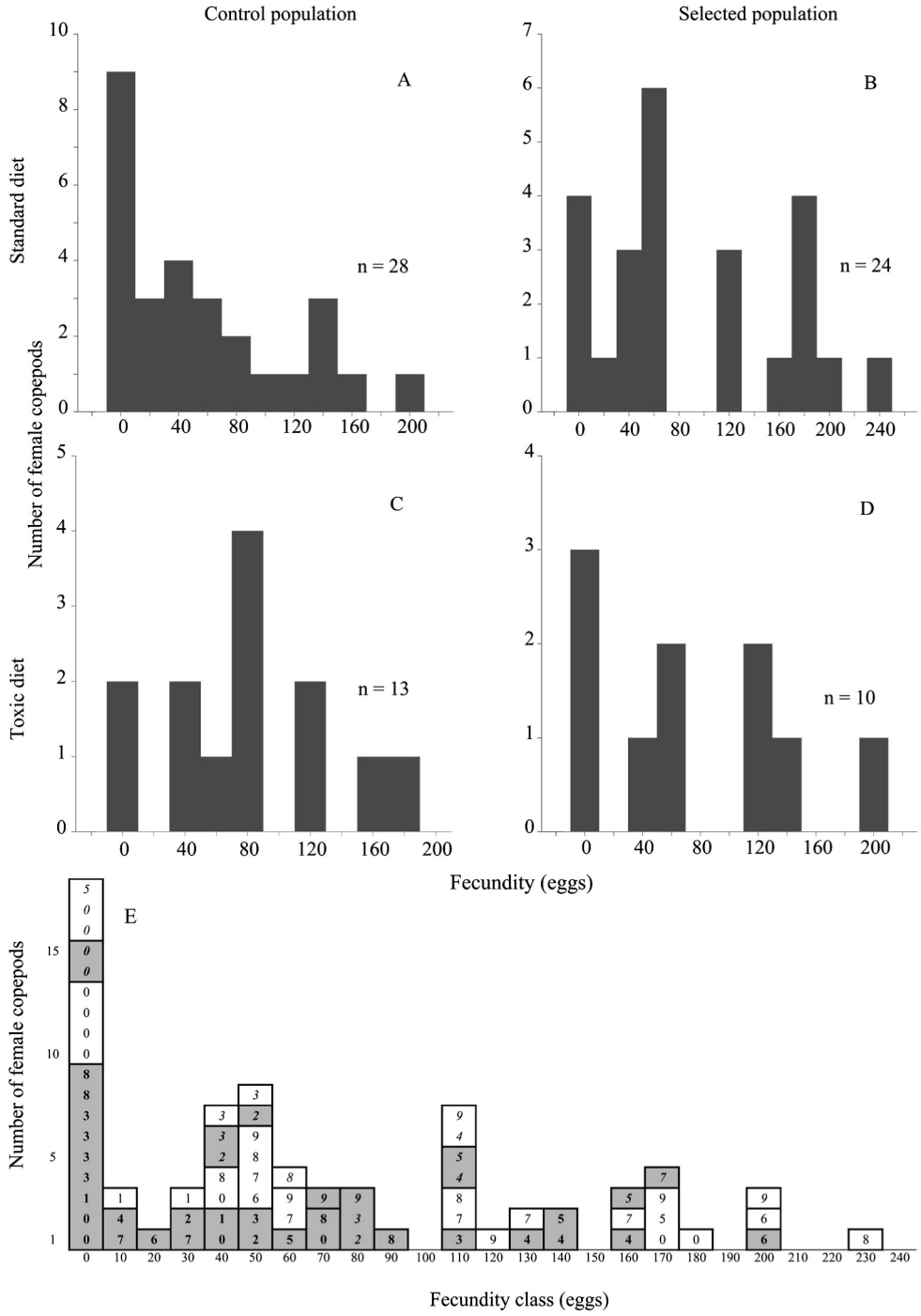

Fig. 1. (A–D) Histograms of fecundity for experimental treatments and (E) combination histogram–stem-and-leaf diagram of pooled data. The *x*-axis in (E) depicts 10-egg bins and can be read as the leading digit(s) of fecundity values for each class. The numbers in the vertical bars constitute the final digit in the fecundity value of each copepod. For example, in the 200-egg class are three copepods producing 206, 206, and 209 eggs, bottom to top. The vertical bars are coded to correspond to experimental treatments: gray fill, control population; no fill, selected population; standard type, standard diet; italic type, toxic diet.

copepodite stage one), and fecundity (egg total), were analyzed for normality using the Kolmogorov–Smirnov test (Zar 1996). A nonparametric analysis of variance (ANOVA) was used (PROC RANK and PROC GLM in SAS v. 9 [SAS Institute]) to test for the effects of source line, or population, and diet on life-history traits. Significance levels were adjusted for the number of simultaneous tests carried out. Regression analysis (model

I) (PROC GLM in SAS v. 9) was used to investigate the trade-offs among the traits further.

Average daily egg production was calculated as the total number of eggs produced by an individual divided by the number of days she was a mature adult. Fecundity was the total number of eggs produced by an individual during the 35-d experiment. Time to maturity or development time was the number of days from hatching to the maturation molt. Average daily egg production rate and time to maturity were further combined into one measure of fitness, similar to λ of McGraw and Caswell (1996). An age-based Leslie projection matrix consisting of n columns representing n days of life was constructed. The average daily egg production of each copepod was distributed over the days of reproduction in the matrix. Because eggs were collected only approximately daily, the observed daily values of egg production could not be used. The principal eigenvalue of this matrix is a measure of fitness that combines development time and daily reproductive rate. It is equivalent to λ mathematically, but it is not lifetime fitness. Instead we call our fitness estimate initial potential fitness (IPF). It represents potential fitness because the eggs were not fertilized. It represents initial fitness because we do not have information beyond the 35th day of the experiment.

Further data analysis—The frequency distributions of fecundity from all treatments suggested to us that discrete reproductive phenotypes were present (Fig. 1). Some of these distributions (e.g., Fig. 1B and when treatments were pooled, Fig. 1E) deviated from normality. Therefore, we pooled the data and created a procedure to examine the discrete nature of the resulting distribution (Fig. 1E). The procedure used the cumulative frequency distribution shown in Fig. 2A, the probability plot. In such a plot, a normal distribution appears as a straight line. This feature of the probability plot has made it useful in many fields (Miesch 1981; Mage 1982; Kim et al. 2005), where it is often used to test for normality. We already had confirmed that our distribution was not normal, but the curve was useful because the inflection points in it indicate the presence of mixing subpopulations (Harding 1949; Miesch 1981). The putative subpopulations are also visible in the stem and leaf diagram as discrete groups (Fig. 1E).

To reveal objectively the divisions among subpopulations, we first smoothed the cumulative frequency distribution by computing a running mean with a window of seven values. The probability values of the empirical distribution were calculated using $p_i = i/n$ for $i = 1...n$, where n = number of observations and i = the rank order of observations. Because the a-axis of the probability plot approaches but never reaches 1.00 (100%), n/n was rounded down to 0.99. We then computed the first derivative of the smoothed curve in a point-to-point fashion. Smoothing allowed us to remove the noise in the derivative calculation, but retain the general trends (Tukey 1977). Other smoothing windows were tried also. They changed the location of inflection points slightly, but had very little effect on the overall results of the procedure (Table 1). Plotting the relative slope (i.e., after being scaled to the highest value of the derivative) of the line against fecundity reveals the local minima and local maxima (Fig. 2B) that correspond to modes and inflection points in Fig. 2A. We then grouped copepods according to the modes and inflection points (Fig 2C).

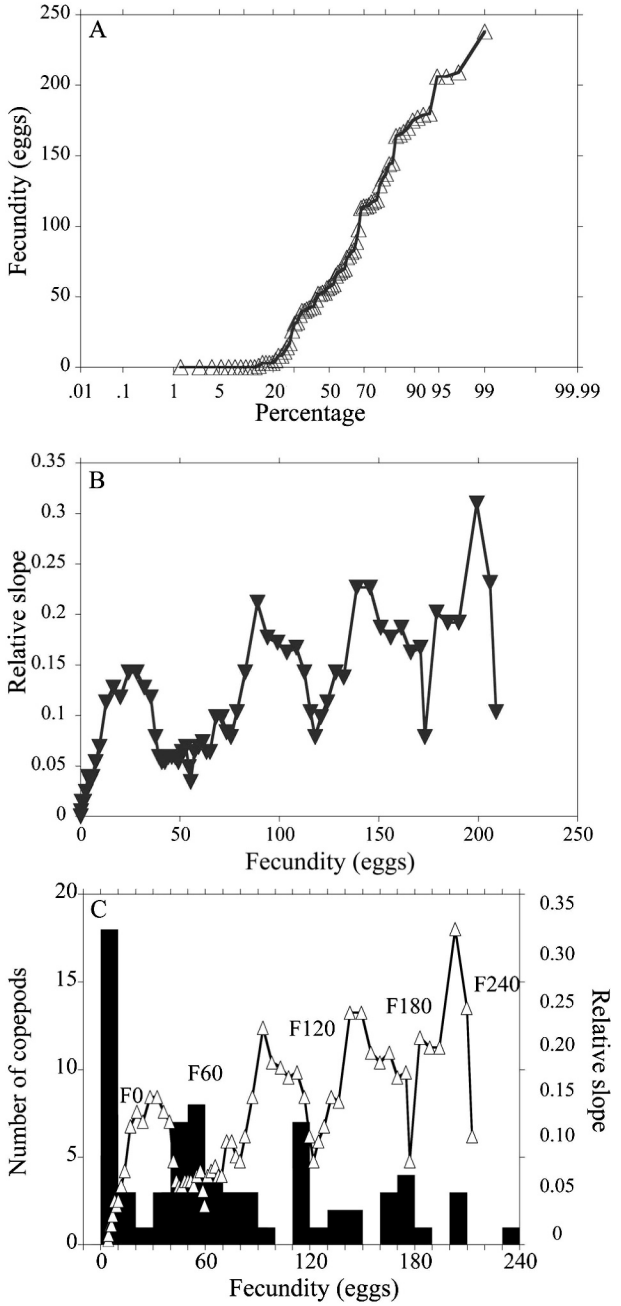

Fig. 2. (A) Cumulative frequency distribution of pooled fecundity data, (B) the relative slope (scaled to the largest value) of the cumulative frequency distribution after it was smoothed with a 7-pt running mean, and (C) the relative slope values overlaid on a histogram of the pooled data. Inflection points in (A) correspond to local maxima in (B) that were used to assign individuals to phenotypes indicated by text (F0, F60, etc.) in (C).

To determine if a discrete distribution adequately described the data, we fit one to the data of each

Table 1. Effect of varying the width of the smoothing window on class (phenotype) identification. After smoothing, the derivative of the empirical frequency distribution was used to assign individuals to presumptive phenotypes. Varying the window has very little effect on the either the number of individuals included in a class or on the median value of fecundity in that class. The number in the F240 class was established by other criteria in addition to the derivative estimate.

Parameter	Phenotype	Width of smoothing window (number of values)			
		0	3	7	11
Boundary revealed by derivative (eggs)	F0	0	0	0	0
	F60	26	25	28	17
	F120	113	108	89	109
	F180	164	158	145	164
	F240	210	210	210	210
Number of copepods in class	F0	22	21	21	21
	F60	30	30	29	30
	F120	12	12	13	13
	F180	10	11	11	10
	F240	1	1	1	1
Median fecundity in class (eggs)	F0	2	1	1	1
	F60	56.5	54.5	53	56
	F120	124	118.5	118	119
	F180	178	177	177	178

experimental treatment (i.e., to the data before pooling). Because we suspected that five modes were present, we chose to fit distributions described by the expansion of $(r + q)^4$ (where r and q represent the relative frequencies of independent mutually exclusive events such that $r + q = 1$), which yields five classes. The discrete distributions were fitted by iteratively adjusting r until a solution that minimized the chi-square test statistic was found. In each case, a statistically significant fit was found. Further, when the distributions from all treatments were pooled, the resulting pooled distribution was found to fit statistically one expected from the sum of the four binomial distributions.

In testing goodness of fit, one can determine only whether observed frequencies deviate significantly from expected hypothetical frequencies (Sokal and Rohlf 1995). Therefore, we cannot claim that our description of the data is the only one possible. We only claim that the statistical evidence supports our interpretation and therefore justifies the analysis that follows.

Once each copepod was assigned to a phenotype, the fitness values of the five phenotypes were calculated and used to test the hypothesis that resistance carries a cost. To test this hypothesis we needed to account for differential survivorship between the two experimental diets, standard and toxic, which IPF does not do. To include differential survivorship, we calculated a weighted IPF (WIPF) as the mean fitness of the survivors in a given phenotype and diet, multiplied by the number of survivors on that diet, divided by the number of survivors of that phenotype on the standard diet. For example, the WIPF of F180 on the toxic diet is 3/8(1.188) = 0.446. It is assumed that the initial frequency of any phenotype in the toxic diet treatment was equal to its initial frequency in the standard diet treatment. Relative WIPFs were calculated by scaling the WIPF of each phenotype to the highest fitness across all phenotypes within a diet. For example, the relative WIPF of F180 on the toxic diet is 0.446/0.729 = 0.611. Fitness, size, time to metamorphosis, time to maturity, and egg production rate of phenotypes were compared using nonparametric ANOVA (PROC RANK and PROC GLM in SAS v.9).

Results

Our original intention in this study was to compare the performances of two copepod populations: a control line (lab-reared but never exposed to toxic algae) and a selected

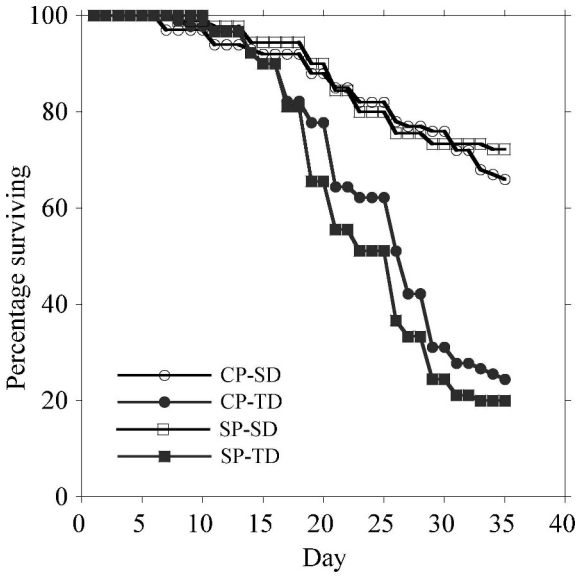

Fig. 3. Survivorship curves for each experimental treatment. CP, control population; SP, selected population; SD, standard diet; TD, toxic diet.

Table 2. Summary of nonparametric ANOVA tests for effect of population and diet on life-history characters in the initial analysis of the data. Alpha levels for tests of female copepods are taken to be significant at the $p=0.0125$ level to correct for four simultaneous tests, males at the $p=0.025$ level.

	Source of variation	Time to metamorphosis (d)			Time to maturity (d)			Adult size (μm)			Egg production rate (egg cop^{-1} d^{-1})		
		df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Female copepods	Population	1	7.78	**0.007**	1	0.03	0.864	1	0.09	0.767	1	2.42	0.124
	Diet	1	3.99	0.050	1	7.84	**0.007**	1	0.07	0.792	1	3.15	0.080
	Interaction	1	1.99	0.163	1	0.91	0.344	1	1.05	0.309	1	0.76	0.387
	n	76			76			69			75*		
Male copepods	Population	1	0.19	0.663	1	0.03	0.861						
	Diet	1	4.43	0.040	1	1.28	0.261						
	Interaction	1	3.16	0.080	1	5.75	**0.019**						
	n	67			67								

* One individual matured but died before the end of the experiment. She had not produced any eggs.

line (lab-reared in presence of the toxic alga for many generations [Colin and Dam 2004]). Surprisingly, our initial data analysis revealed very little difference between the two lines. Survivorship was similar for both populations (Fig. 3). In the toxic diet 24% of the control population and 20% of the selected population survived, whereas approximately 70% of each survived in the standard diet. Although the toxic diet significantly increased the time to maturity of females ($F_1 = 7.84$, $p = 0.007$), population affected only the age at metamorphosis ($F_1 = 7.78$, $p = 0.007$). Neither population ($F_1 = 0.09$, $p = 0.767$) nor diet ($F_1 = 0.07$, $p = 0.792$) affected adult size or average daily egg production. Population and diet interacted significantly in male copepods ($F_1 = 5.75$, $p = 0.019$) (Table 2).

In addition, when we examined the relations among life-history traits we found no diet- or population-related differences. For example, regressing daily egg production rate on time to maturity showed a significant relation in all treatments (Fig. 4), but the slopes were similar among treatments ($F_3 = 1.34$, $p = 0.269$). Adult size showed no significant relation to time to maturity (not illustrated). Time to metamorphosis did show significant relations with time to maturity, but they are not shown because the relation is trivial. Time to metamorphosis equals approximately half of time to maturity. Life-history traits, survivorship, and regression relations were all similar among treatments so we pooled populations and re-examined the data in a different way.

This further data analysis revealed the patterns related to resistance that are the primary focus of the rest of this report. Because some distributions (e.g., Fig. 1B) did not fit a normal distribution (Kolmogorov–Smirnov test statistic $D = 0.198$, $n = 24$, $p > 0.05$) and because there appeared to be discrete classes of fecundity in all treatments (Fig. 1), we chose to fit the discrete distribution described by the expansion of $(r + q)^4$, which yields five classes. A significant fit for all treatments ($p < 0.05$; $\chi^2_{0.05} = 9.488$, $\nu = 4$) was found (control–standard: $r = 0.762$, $\chi^2 = 1.30$, $\nu = 4$; control–toxic: $r = 0.762$, $\chi^2 = 5.89$, $\nu = 4$; selected–standard: $r = 0.602$, $\chi^2 = 6.27$, $\nu = 4$; selected–toxic: $r = 0.698$, $\chi^2 = 0.658$, $\nu = 4$). After the distributions from all treatments were pooled, the resulting pooled distribution was found to fit statistically one expected from the sum of the four binomial distributions ($\chi^2 = 6.05$, $\nu = 4$).

Once a discrete distribution that described all of the data was known, we could treat each class as a putative phenotype. But first we had to assign individuals objectively to one of the putative phenotypes. To accomplish this we used the probability plot shown in Fig. 2A. The first derivative of the probability plot (Fig. 2B) revealed local minima (corresponding to modes in the original data) at 0, 57, 118, and 173 eggs and local maxima (corresponding to inflection points in Fig. 2A) at 28, 89, and 145 eggs. Because the distribution is sparse at the high-fecundity end, and therefore the derivative calculation is likely to be unreliable, we considered the final inflection point to occur between 209 and 210 eggs. The procedure resulted in five phenotypic classes: 0–28, 29–89, 90–145, 146–209, and 210–238 eggs. The phenotypic classes were named for the approximate modal or median fecundity value of each class: F0, F60, F120, F180, and F240 (Fig. 2C). This organization of the data is appealing because it is consistent with the binomial distribution that describes the data; that is, the classes are approximately symmetrical. It is also appealing because it can be explained by well-known biological mechanisms, i.e., segregating genetic elements.

With the data reorganized, we then re-examined fitness and other life history data and compared the results among phenotypes. Phenotype significantly affected IPF ($F_4 = 158.47$, $p < 0.001$), but diet did not ($F_1 = 3.12$, $p = 0.082$) (Table 3). Recall that WIPF includes differential survivorship between diets. Recall also that costs and advantages are revealed by comparisons among phenotypes within a diet. Comparing WIPF values within the standard diet treatment shows that F240 had the highest fitness, whereas in the toxic diet treatment, F120 had the highest fitness (Table 3).

Subtracting the relative WIPF of each phenotype from the relative WIPF of F240 (the highest relative WIPF) on the standard diet reveals the costs of resistance (Fig. 5). High costs are borne by the phenotype F0, which had a 46% reduction in fitness ($1.000 - 0.540 = 0.460$). The costs

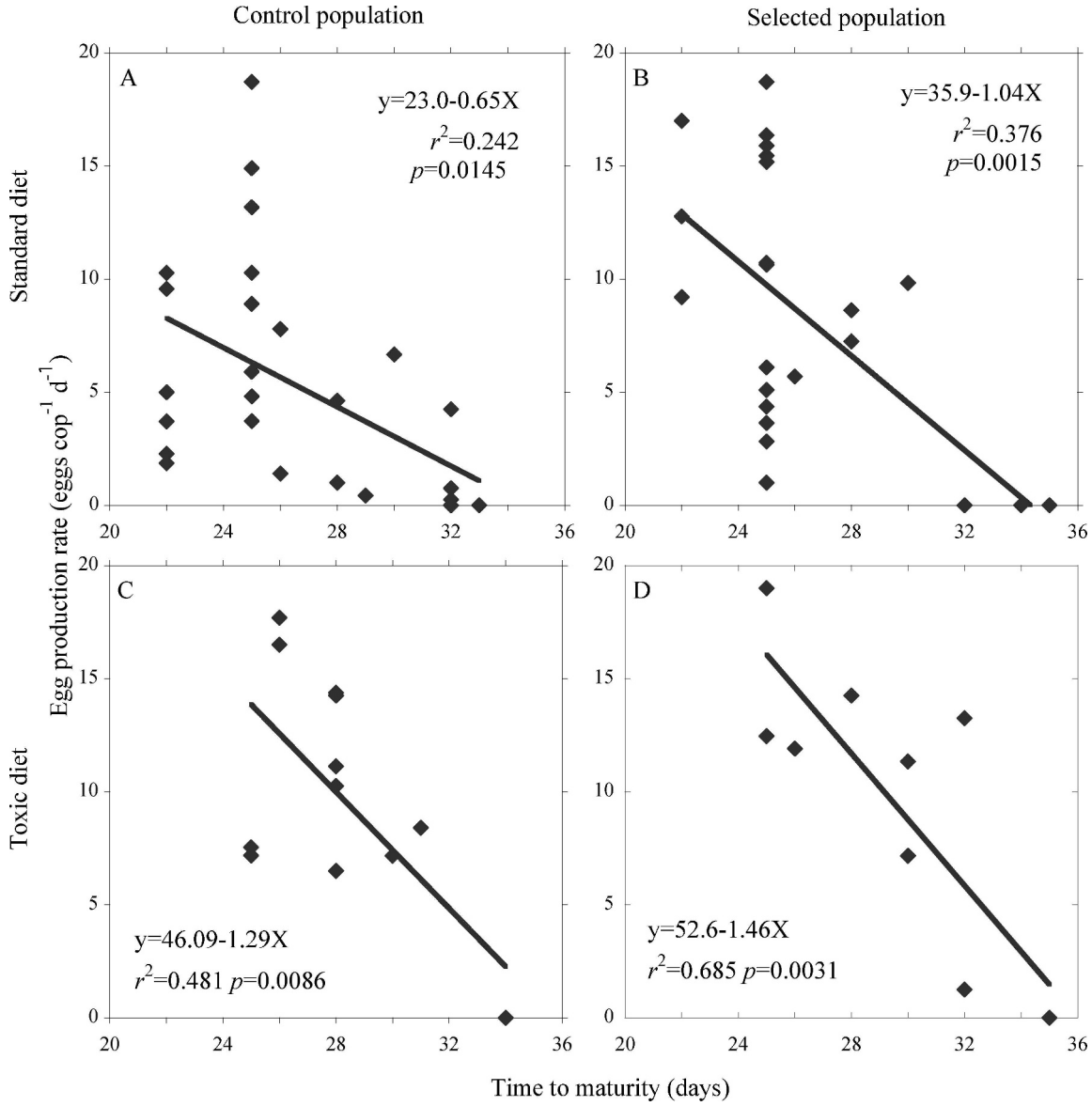

Fig. 4. (A–D) Regression relations between daily egg production rate and development time in each of four experimental treatments. Individual points represent the average daily egg production rate of one copepod. Egg production rate was calculated as the total number of eggs divided by the number of days that an individual was mature during the experiment.

experienced by other phenotypes are relatively low (2% to 7% reduction). The fitness advantages of resistance are determined in a similar manner using the toxic diet treatment. The greatest fitness advantage was enjoyed by the phenotype that we designated as F120 (Table 3, Fig. 5). For example, it had a 91% advantage over F0.

Nonparametric ANOVA showed significant differences among phenotypes in several life-history characters in addition to fitness (Table 4) (time to metamorphosis: $F_4 = 4.27$, $p = 0.0004$; time to maturity: $F_4 = 16.61$, $p < 0.0001$; daily egg production: $F_4 = 60.0$, $p < 0.0001$). Moreover, there are significant trends across phenotypes in several characters wherein F0 had slow development and low egg production and F180 and F240 had fast development and high egg production. Adult size did not show significant differences among phenotypes ($F_4 = 1.25$, $p = 0.287$) but also showed signs of a trend (Table 4).

Discussion

The results of this study are the first to reveal the presence of discrete reproductive phenotypes in a copepod. They also reveal for the first time the individual variation in PSP toxin resistance in a copepod. They provide insight into the fitness advantage provided by resistance as well as the costs borne by resistant individuals in a nontoxic environment. In addition, the data suggest that resistance is a simple genetic system (few genes) and that it probably involves altering the balance of energy during a copepod's growth, development, and reproduction.

Table 3. Fitness of each phenotype in two diets. Initial potential fitness (IPF) was calculated from fecundity and time to maturity by a Leslie projection matrix. Weighted IPF (WIPF) weights the fitness of each phenotype according to the survivorships in the two diets. Relative WIPF scales fitness values to the highest value within a diet. The superscripts accompanying IPF values indicate statistical differences among phenotypes within a diet. 95% confidence intervals (CI) for IPF, WIPF, and relative WIPF are indicated by the mean ± CI. CIs cannot be calculated for F240. CIs for F0 are wide because of the large number of zero fitness values in that class.

		Initial potential fitness (IPF)		Weighted IPF		Relative WIPF	
		Diet					
Phenotype		Standard	Toxic	Standard	Toxic	Standard	Toxic
F0	Mean	0.658±1.12[a]	0.210±1.30[a]	0.658±1.12	0.066±0.353	0.540±0.919	0.090±1.314
	n	16	5				
	SD	0.527	0.469				
	Median	1.016	0.000				
F60	Mean	1.138±0.027[b]	1.140±0.036[b]	1.138±0.027	0.600±0.019	0.934±0.022	0.823±0.050
	n	19	10				
	SD	0.013	0.016				
	Median	1.139	1.137				
F120	Mean	1.181±0.026[c]	1.167±0.022[c]	1.181±0.026	0.729±0.014	0.969±0.021	1.000±0.028
	n	8	5				
	SD	0.011	0.008				
	Median	1.173	1.171				
F180	Mean	1.192±0.024[cd]	1.188±0.043[d]	1.192±0.024	0.446±0.016	0.978±0.020	0.611±0.030
	n	8	3				
	SD	0.01	0.01				
	Median	1.189	1.189				
F240	Mean	1.219[d]	0.000	1.219	0.000	1.000	0.000
	n	1	0				
	SD						

Advantage of resistance—The greatest fitness advantage in the presence of the toxic diet was enjoyed by the phenotype that we designated as F120. Its advantage stemmed from moderate fecundity and maximum relative survivorship (Table 3). The advantage of the F120 phenotype may be a case of overdominance or heterozygote advantage. Heterozygote advantage in this trait would prevent the fixation of resistance alleles. So, no matter how often or how severe the repeated toxic blooms, *A. hudsonica* populations could not become fixed at the loci conferring resistance. We believe that this fact probably explains why we apparently have not been able to drive our laboratory populations to fixation, even with many generations of intense selection (unpubl. data).

Colin and Dam (2002*b*, 2004) compared the fitnesses of populations fed *Alexandrium* sp. and found that those populations that were historically exposed to toxic algae performed better than those naïve to it. In their experiments, they used pooled groups of copepods from each population, and so they were comparing population mean responses. Our results suggest the mechanism by which the populations differed: in frequency of resistant phenotypes. Populations that are often exposed to blooms of toxic *Alexandrium* sp. would be expected to have a greater proportion of resistant phenotypes in them than would naïve populations.

Cost of resistance—Resistance alleles that carry a large cost in the absence of toxin would slow or prevent the fixation of resistance alleles in a population. Our results indicate that there is a substantial cost to resistance for one phenotype. F0 shows large fitness losses relative to other phenotypes on the standard diet even while it has a slight fitness advantage over F240 on the toxic diet (Fig. 5). F0 also shows substantial reductions in fitness relative to most other phenotypes on the toxic diet. Such a pattern of relatively low fitness in both toxic and benign environments indicates that this phenotype would not be common in a natural population under any circumstances because it would be selected against. However, our experiment tested only two environments. It is possible that apparently maladaptive phenotypes in these experimental treatments could very well be adaptive in other circumstances.

Other phenotypes also experience losses of fitness on the standard diet (Fig. 5), but the costs are relatively small (2% to 7%), especially when compared with the fitness gains they experience on the toxic diet. These phenotypes would probably be present at all times in wild populations—but in varying proportions—leading to a polymorphism. A polymorphism of resistance in a population could confuse the interpretation of typical experiments testing the effects of the toxic alga on copepods (selective grazing experiments, for example). In those experiments, groups of copepods are typically pooled in an experimental container. Such groups would yield different answers in the same or repeated experiments if they contained different proportions of resistant phenotypes.

Simple genetic mechanism—It is somewhat problematic that in the initial data analysis our so-called selected line

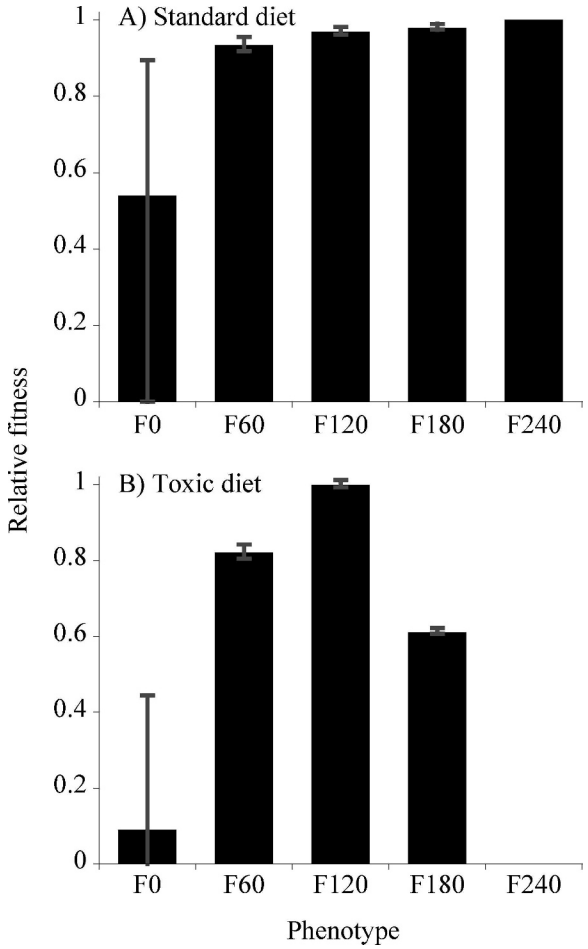

Fig. 5. Relative weighted initial potential fitness of each phenotype on two diets. (A) The fitness costs of resistance are revealed on the standard diet. Comparing relative fitnesses among phenotypes on the standard diet shows that F0 had a 46% reduction in fitness relative to F240. The other phenotypes had costs between approximately 2% and 7%. (B) The fitness advantages of resistance are revealed on the toxic diet, where, for example, F120 has a 39% advantage over F180 (1.00 − 0.611 = 0.389). Error bars indicate the range of relative fitness estimates for each phenotype.

showed no greater survivorship than did the control line (Fig. 3). We believe that the reason for this lies in the two generations of recombination that were allowed the selected line before the beginning of the experiment. In just two generations, the selected line had regenerated enough of the gene combinations of the control line to be indistinguishable from it, at least with respect to total mortality (Fig. 3). If such recombination took place, the resistance first observed by Colin and Dam (2002*b*) is probably controlled by a simple genetic mechanism. Indeed a simple mechanism is consistent with the results of a genetic selection experiment (Colin and Dam 2004) in which a line exposed to toxic *A. fundyense* diverged toward resistance to toxin in just two or three generations. Other data are consistent with a simple genetic mechanism as well. For example, the discrete distributions that we fit to our data are consistent with what is expected from just one or a few segregating genes. Although the data are not sufficient to determine how many genes confer resistance, they are consistent with a simple mechanism.

Resistance alleles are common—Referring again to the initial data analysis, there were few differences between the control population and the selected population in this experiment (Table 2, Fig. 3). One might conclude that our so-called selected line was not selected for resistance at all. However, at least one life-history character was clearly affected by population (time to metamorphosis in females) and another showed a significant interaction of population and diet (time to maturity in males) (Table 2), indicating some effect of selection. An alternative conclusion to "no selection" is that resistance alleles are common, at least in our laboratory populations. Resistance alleles could be maintained—even in the absence of toxin—by moderate heterozygote fitness on the standard diet, such as may be present in the F120 phenotype (Table 3). It is impossible to say from the data at hand how common resistance alleles might be in natural populations. If they are as common as our data suggest, then all populations of *A. hudsonica* harbor the genetic diversity to respond to blooms of toxic *Alexandrium* sp.

Broader implications of resistance—The mechanism of resistance is not yet known, but whatever it is, it appears to influence development. Females had longer mean times to maturity on the toxic diet than on the standard diet (Table 2, Table 4). From our results we can conclude that this difference is due to inherent characters of the copepods. It does not result from direct effects of the toxin. Recall that the life-history indices recorded are only those of surviving copepods. Recall also that on the toxic diet the survivors are, by definition, resistant. Resistant copepods survive on the standard diet too, where some copepods also develop late (>25 d) (Fig. 4). Therefore, the significant difference between the mean time to maturity of female copepods on the standard diet and the mean development time on the toxic diet (Table 2, Table 4) is due to the presence of early-developing copepods in the standard diet treatment. Early developers are either absent or very rare in the toxic diet treatment (Fig. 4). Thus we conclude that the least-resistant copepods are also the fastest developers, whereas more-resistant types are slower developers.

Slow-developing copepods also appeared to be food limited even in the presence of abundant, nontoxic food. For example, F0 phenotypes produced less than one egg per day (Table 4). It follows that starvation may have delivered the coup de grâce in the toxic diet treatments of our experiment. That is, copepods susceptible to the toxic alga died because they could not feed or assimilate food effectively. Dutz (1998), in a study of ingestion and egg production in *A. clausi*, suggested that saxitoxin interferes with assimilation. We hypothesize, then, that the mechanism that confers toxin resistance may also provide protection against starvation, though by what means remains to be understood.

Table 4. Phenotype-specific mean values for copepod life-history characters by diet. Small letters (*a,b,c*) at the head of each column indicate statistically significant effects of phenotype (*a*), diet (*b*), or interaction of both (*c*) on the life-history character as revealed by nonparametric ANOVA. No letter indicates nonsignificance (e.g., size). Significance levels were adjusted to account for the number of simultaneous tests, $p=0.05/4=0.0125$.

		Time to copepodite stage one (d) *a*		Time to maturity (d) *ab*		Size, prosome length (μm)		Daily egg production rate (eggs per copepod) *abc*	
Phenotype		Standard	Toxic	Standard	Toxic	Standard	Toxic	Standard	Toxic
F0	Mean	18.13	21.60	30.88	34.00	723.27	714.43	0.90	0.33
	SD	2.00	3.78	2.83	1.22	25.24	42.69	1.38	0.75
	n	16	5	16	5	13	5	16	5
F60	Mean	15.74	16.90	25.47	28.70	737.24	731.64	5.90	10.70
	SD	1.56	2.51	2.48	2.36	43.65	31.01	2.55	3.25
	n	19	10	19	10	18	9	19	10
F120	Mean	15.25	16.00	23.88	27.00	740.91	738.41	11.30	15.18
	SD	2.31	2.00	1.55	1.41	20.95	34.68	1.18	1.58
	n	8	5	8	5	8	5	8	5
F180	Mean	16.00	16.67	24.63	25.67	751.90	769.39	17.57	19.63
	SD	0.00	1.15	1.06	0.58	27.49	14.14	2.25	1.28
	n	8	3	8	3	8	2	8	3
F240	Mean	14.00		22.00		714.43		18.31	
	SD								
	n	1		1		1		1	

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