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EVOLUTIONARY CONSTRAINTS AND THE MAINTENANCE OF INDIVIDUAL SPECIALIZATION THROUGHOUT SUCCESSION

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Constraints on life-history traits, with their close links to fitness, are widely invoked as limits to niche expansion at most organizational levels. Theoretically, such constraints can maintain individual specialization by preventing adaptation to all niches available, but empirical evidence of them remains elusive for natural populations. This problem may be compounded by a tendency to seek constraints involving multiple traits, neglecting their added potential to manifest in trait expression across environments (i.e., within reaction norms). By replicating genotypes of a colonial marine invertebrate across successional stages in its local community, and taking a holistic approach to the analysis of ensuing reaction norms for fitness, we show the potential for individual specialization to be maintained by genetic constraints associated with these norms, which limit the potential for fitness at one successional stage to improve without loss of fitness at others. Our study provides new insight into the evolutionary maintenance of individual specialization in natural populations and reinforces the importance of reaction norms for studying this phenomenon.

KEY WORDS: **Among-individual niche variation, competition, constraints, fitness, life-history traits, reaction norms.**

Explanations of successional diversity often rely on life-history trade-offs that allow competitors to coexist in spatially structured habitats (Amarasekare 2003). For example, early-successional species should typically excel when resources are abundant but do relatively poorly when resources are scarce, whereas late-successional species should be the opposite—that is, worse at exploiting resource-rich conditions but better at tolerating resource-poor ones (Rees et al. 2001). Classic theory (e.g., MacArthur and Wilson 1967) attributes these phenotypes to environment-dependent selection that favors the greater productivity of weaker competitors in uncrowded, resource-rich sites opened by disturbance, but favors the greater efficiency with which stronger competitors convert resources to reproductive output as crowding and competition increase with succession. The problem, however, is that this interplay of life history, succession, and disturbance is well-established theoretically and supported by laboratory experiments, but field evidence has proven elusive (Clark 2010; Vellend 2010).

Why might this be? One possibility is that intraspecific variation swamps species-level differences throughout succession, but a tendency for ecologists to treat conspecifics as equivalent has seen it often reduced to sampling error—an approach warranted only if such variation is rare, weak, or ecologically irrelevant (Bolnick et al. 2003). From an evolutionary perspective, the importance of intraspecific variation is unquestioned. Individual fitness is the trait "seen" by natural selection; a population's mean fitness improves in response according to how much individual fitness varies genetically, and other traits evolve adaptively when they have causal links to fitness (Falconer and Mackay 1996). The puzzling fact that heritable variation in fitness (or life-history traits that closely relate to it) often persists in nature when selection should deplete it, however, may mean that constraints on the

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selection response also occur (Walsh and Blows 2009). Like their role in species diversity, such constraints are thought to maintain within-population diversity by limiting the niches of individual genotypes, seeing them often invoked to explain nature's lack of "Darwinian demons" (generalists that maximize all components of fitness simultaneously) and the existence of individual specialization (Futuyma and Moreno 1988; Agrawal et al. 2010). These evolutionary alternatives may be described in terms of the among- and within-individual components of a population's total niche, with among-individual niche variation (reflecting genetic variation in resource use) expected to be large relative to the average within-individual niche width in populations of specialists, but comparatively small in populations of generalists (Van Valen 1965; Roughgarden 1972; Bolnick et al. 2003). Understanding the evolutionary maintenance of specialization may thus be a matter of understanding among-individual niche variation.

Although recent coverage of this issue emphasizes constraints involving trade-offs between different life-history traits or fitness components (Poisot et al. 2011), constraints can also affect the same trait expressed in different environments (Fry 2003; Agrawal et al. 2010). Constraints of this kind can be illustrated via reaction norms (Woltereck 1909), which map the trait variation of genotypes to environment and are used to study phenotypic plasticity or, when studying fitness components, environmental tolerance (Lynch and Gabriel 1987). There has been some debate, however, over different modeling approaches that seek to explain the same underlying biology, but partition trait variation differently so that model parameters mean somewhat different things (de Jong 1995; Via et al. 1995). On one hand, the character-state approach treats norms as sets of trait values expressed in discrete environments, with constraints showing as a lack of genetic variation within environments, or as unfavorable genetic correlations across them such that adaptation to some environments can improve only at the cost of adaptation to others. On the other hand, the polynomial approach treats norms as functions of a continuous environmental range, with constraints showing in the genetic variances or correlations of polynomial coefficients. For example, an unfavorable correlation between the elevation (mean phenotype over an environmental range) and slope (change in phenotype over that range) of polynomial norms may imply that adapting to a broad range of environments tends to lower fitness in any single one, to the point that a jack-of-all trades masters none (Van Tienderen 1991). Both approaches offer complementary insights into the kind of constraint that underpins most theory for the evolution of specialization (Lynch and Gabriel 1987; Van Tienderen 1991; Gomulkiewicz and Kirkpatrick 1992). If the same genotype does not perform best in all environments, then genotype–environment interactions in fitness can potentially favor individual specialization in spatially structured habitats (Futuyma and Moreno 1988).

How easily the concepts of among-individual niche variation and specialization translate to a reaction-norm perspective makes reaction norms, and principles about their evolution, natural tools for exploring the role of fitness constraints in successional diversity. From this perspective, reaction norms are traits much like any other: they must vary genetically to evolve and, if unconstrained, should do so until a population has maximal fitness throughout its environmental range and such variation is exhausted. This process amounts to the adaptive expansion of within-individual niche width (Lynch and Gabriel 1987; Whitlock 1996). If evolutionary constraints exist, however, the population may reach equilibrium with a suboptimal norm and comprising a mix of genotypes that are each most fit over some part of this range (Gomulkiewicz and Kirkpatrick 1992; Gavrilets and Scheiner 1993). In the case of reaction norms spanning succession, such genotypes are readily seen as specialists in successional niches maintained by disturbance. To our knowledge, this way of exploring among-individual niche variation in natural populations has received surprisingly little attention from empiricists. We studied individual variation in reaction norms relating the reproductive output of a colonial invertebrate to succession in its local epifaunal community. Such communities are predominantly sessile animals for which space is limiting, but often made available by disturbance (Connell and Keough 1985). As newly-open patches are invaded by a succession of organisms, with early colonizers overgrown or outlived by subsequent arrivals, the interplay of these processes structures the community into a mosaic of successional stages (Edwards and Stachowicz 2010). In this ecological setting, we tested whether constraints on reaction-norm evolution may slow or prevent our study population from maximizing fitness across the successional range that it typically encounters. In doing so, we shed new light on the potential evolutionary maintenance of individual specialization.

Materials and Methods

STUDY ORGANISM AND SITE

The encrusting bryozoan, *Hippopodina iririkiensis* (Tilbrook 1999; named by genus hereafter), has a near-circumtropical distribution, growing on submerged surfaces as sheet-like colonies of modular subunits (zooids). The primary module is a feeding zooid (a feeding-organ, gut, and hermaphroditic gonads enclosed in calcified walls) that reproduces sexually and by asexual budding. Some feeding zooids become functionally female and, when fertilized, brood embryos in conspicuous brood-chambers (used repeatedly throughout the maternal zooid's lifetime) until releasing them as swimming larvae that soon settle and form new colonies. *Hippopodina* is common at our field site (Manly Boat Harbour, Queensland, Australia), where submerged surfaces host diverse epifaunal communities.

SAMPLING OF COMMUNITY SUCCESSION

To sample succession at our field site, we bolted small (0.01 m^2) PVC plates to the undersides of larger (0.25 m^2) panels submerged below floating docks, deploying 80 plates on five panels around our site initially and a replicate array 8 weeks later. Plates were roughened to encourage the settlement of propagules. Six weeks later, all 160 plates were brought to the laboratory, supplemented by 80 more that had been immersed at our field site but remained free of epifauna. At this point, the communities founded at different times represented three key stages of development (sensu Mouquet et al. 2003): a colonization stage with residents yet to establish (provided by minimal coverage of space at 0 weeks), a nonsaturated stage with residents interacting too weakly for competitive exclusion (provided by ∼50% coverage at 6 weeks), and a saturated stage with residents interacting strongly enough for exclusion to occur (provided by ∼100% coverage at 14 weeks).

SAMPLING OF FOCAL GENOTYPES

To sample focal genotypes, we let larvae from wild colonies settle naturally on sheets of roughened acetate, fixed to the undersides of panels submerged below floating docks. Panels were well spaced initially to capture representative patterns of genetic variation in the population. After ∼2 weeks of settlement, we cleared sheets of all settlers but *Hippopodina*, consolidated them on panels hung together (to minimize environmental effects that can inflate genetic estimates) and removed new invaders for months thereafter to give focal colonies ample space to grow. We then brought 20 colonies, each ∼6 cm across, on their acetate to the laboratory for fragmentation and transplantation into the preestablished communities described earlier. Since the limited dispersal of *Hippopodina* larvae (which swim <1 hour before settling) means that neighboring colonies might be siblings, we carefully chose nonneighboring colonies from different acetate sheets. None was reproductive at this point.

REACTION NORMS FOR FITNESS ACROSS SUCCESSIONAL STAGES

In the laboratory, we cut 12 fragments from each focal genotype, ensuring all were similar in size (∼1 cm^2), shape, and ratio of mature zooids to colony margin (the budding site). We glued each fragment via its acetate base into a patch of similar size and shape cleared on each plate, assigning 4 fragments per genotype to each successional stage. Plates were photographed to record initial fragment sizes and positions (for later identification) before returning plates to the field. There, they were bolted to 40 panels in a split-plot design, replicating each genotype twice per successional stage on each of two panels. Panels were submerged randomly below floating docks and tended weekly by removing invaders from the youngest communities to hold them at early succession. After 6 weeks, with focal colonies visibly reproductive and/or outgrowing their plates, we retrieved them for measurement. First, we photographed each colony to record its final size (using ImageJ; http://rsb.info.nih.gov/ij). Next, we sampled a radial segment of constant size and recorded the density of brood chambers within it. Finally, we multiplied this density by colony size for a measure of fitness reflecting colony-wide reproductive output. We added 1 to densities beforehand to base the fitness of infertile colonies on size alone and square-root transformed combined data afterward to improve normality. Fitness scores were mean-centered and expressed in units of standard deviation (SDU) for analysis.

STATISTICAL ANALYSES OF REACTION NORMS

Data were analyzed with 2 complementary models (fitted via REML in PROC MIXED of SAS 9.3). First, we modeled reaction norms using the character-state approach, treating fitness as a set of character-states expressed at discrete successional stages (early, mid, and late) defined by weeks of community development (0, 12, and 20 weeks, respectively, given the 6-week deployment of focal colonies and continual clearing of early-stage communities). Succession was modeled as a fixed effect at the population-level (fitting a mean character-state norm) and a random effect at the level of genotype (fitting character-state norms to genetic individuals). This estimated the total (or broad-sense) genetic variance of each character-state, plus the genetic correlations between pairs of states, based on random deviations of genotypic norms from the population mean (Littell et al. 2006).

Second, we modeled *Hippopodina* reaction norms using the polynomial approach, treating fitness as a regression function of a continuous successional range. We fitted a linear regression (defined by elevation and slope) only, for simplicity and because raw norms (Fig. S1) suggested most, if not all, approximated this shape. Mirroring our character-state model, regression coefficients were modeled as fixed at the population-level (fitting a mean polynomial norm) and random at the level of genotype (fitting polynomial norms to genetic individuals), estimating the total genetic variance of each coefficient, plus genetic correlations between coefficients, based on random deviations of genotypic norms from the population mean (Littell et al. 2006). To estimate coefficients on similar scales, weeks of community development were mean-centered and expressed in SDU (matching fitness scores) before analysis (Martin et al. 2010).

The models were otherwise identical: both included panel-within-genotype as the only other random effect (preliminary tests showed that effects of succession did not vary significantly at this level; all $P \geq 0.10$) and estimated residual variances by successional stage. Both also included initial size as a fixed covariate to adjust for its possible fitness consequences, once preliminary tests found it not to vary at any level that precluded doing so (all $P \geq 0.13$). We tested the significance of fixed effects using F-tests

and of random effects using likelihood-ratio tests (LRTs). These were two-tailed when comparing correlations to 0, and one-tailed when comparing variances to 0 or correlations to 1. We visualized reaction norms using best linear unbiased predictors (BLUPs) of genotypic values for character-states or polynomial coefficients, extracted from models and reexpressed on the original data scale.

Finally, we explored the multivariate structure of heritable variation in reaction norms by reparameterizing each model to estimate **G**, the genetic covariance (rather than correlation) matrix of character-states or polynomial coefficients, and using factor-analytic modeling to find its rank (i.e., how many independent dimensions of variance it represented; Hine and Blows 2006). Because reaction norms will also be evolutionarily constrained when multiple correlations among parameters, or lack or variance in any, cause either of these matrices to be singular (i.e., not full rank; Via et al. 1995), such modeling offers an alternative **G**-wide test of constraint that is more comprehensive than testing genetic variances and correlations one-by-one (Kirkpatrick 2009; Walsh and Blows 2009; Conner 2012).

We first modeled each **G** as unstructured, estimating all elements independently, before fitting a set of nested models that were constrained to estimate **G** with one dimension less at a time, and using LRTs to find when a reduction in rank caused significant loss of model fit (Hine and Blows 2006). We then viewed the statistically supported dimensions of **G** as the principal components (PCs) of the appropriately reduced matrix identified by our tests. The eigenvalue of each PC describes the amount of variance in **G** accounted for, whereas PC loadings describe the joint pattern of genetic correlation among reaction-norm parameters defining it. Because PCs are orthogonal, they may be interpreted as the combinations of parameters that can respond to selection independently (Kirkpatrick 2009).

Results

Hippopodina incurred lower fitness when invading communities later in succession. For character-state norms, this showed as significant declines in the population mean (black line in Fig. 1A) by 1.35 SDU from early- to mid-succession ($F_{(1,\,19)} = 74.82$, $P < 0.01$) and by 0.51 SDU from mid- to late-succession ($F_{(1,\,19)} = 26.63$, $P < 0.01$). Some disparity in these declines is likely due to the uneven spacing of our successional stages. For polynomial norms, it showed as a significantly-negative mean slope (black line in Fig. 1B) of −0.91 SDU per unit of community development over this range ($F_{(1,\,19)} = 122.13$, $P < 0.01$).

HERITABLE VARIATION IN CHARACTER-STATE NORMS

Character-state reaction norms varied significantly among genotypes throughout succession, with heritable variation declining 10-fold from early to late stages (Fig. 1A; Table 1A). The cross-stage genetic correlation of fitness was weakly positive between adjacent stages and weakly negative between nonadjacent ones (Fig. 1A, inset). Each correlation was indistinct from 0 but significantly less than 1 (Table 1A), indicating that succession modified the fitness rankings of genotypes to the point that performance at one stage poorly predicted performance at others (Robertson 1959; Fry 1996).

As a *post hoc* test of whether different genotypes performed best at different successional stages, we chose the five best-performing genotypes from early- and late-succession, respectively, and compared their BLUPs jointly at each stage using a random-effect contrast (implemented in the main model to incorporate uncertainty in all values; see Littell et al. 2006). As implied in Figure 1A, these subsets of genotypes reversed their relative fitness throughout succession, differing significantly at each extreme ($t_{(22)} \geq |3.21|$, $P < 0.01$) but performing similarly well in between ($t_{(65)} = -1.12$, $P = 0.27$). These tests are meant simply to clarify the pattern of variation in our sample; whereas further sampling of our study population could in principle uncover a universally superior genotype, they nonetheless suggest that such a genotype is unlikely to be common.

HERITABLE VARIATION IN POLYNOMIAL NORMS

Polynomial norms varied significantly among *Hippopodina* genotypes in both elevation and slope (Fig. 1B and Table 1B), indicating heritable variation in both mean fitness and the degree of environmental tolerance over that range. The genetic correlation between elevation and slope was significantly negative (Table 1B), implying that genotypes performing well on average (signaled by high elevation) were worst-affected by succession (signaled by highly negative slope), whereas genotypes performing poorly on average were more tolerant of this change (Fig. 1B, inset).

It is worth noting here that polynomial and character-state norms are related mathematically in such a way that the variances and covariance of elevation and slope are confounded in the variances of character-states, whereas the latter differ across environments (as implied in Table 1A) whenever elevation and slope are correlated (Lynch and Walsh 1998). Effectively, this means our analysis of polynomial norms cannot test whether fitness rankings change (i.e., reaction norms cross) across succession, any more than our analysis of character-state norms tests whether a genotype's mean fitness and tolerance of succession are related. Together, however, our analyses illustrate the complementarity of these approaches for understanding reaction-norm variation.

Fitness varied significantly among panels in both the character-state model ($\mathrm{LRT}_{(1)} = 5.17$, $P = 0.01$) and the polynomial one ($\mathrm{LRT}_{(1)} = 3.03$, $P = 0.04$).

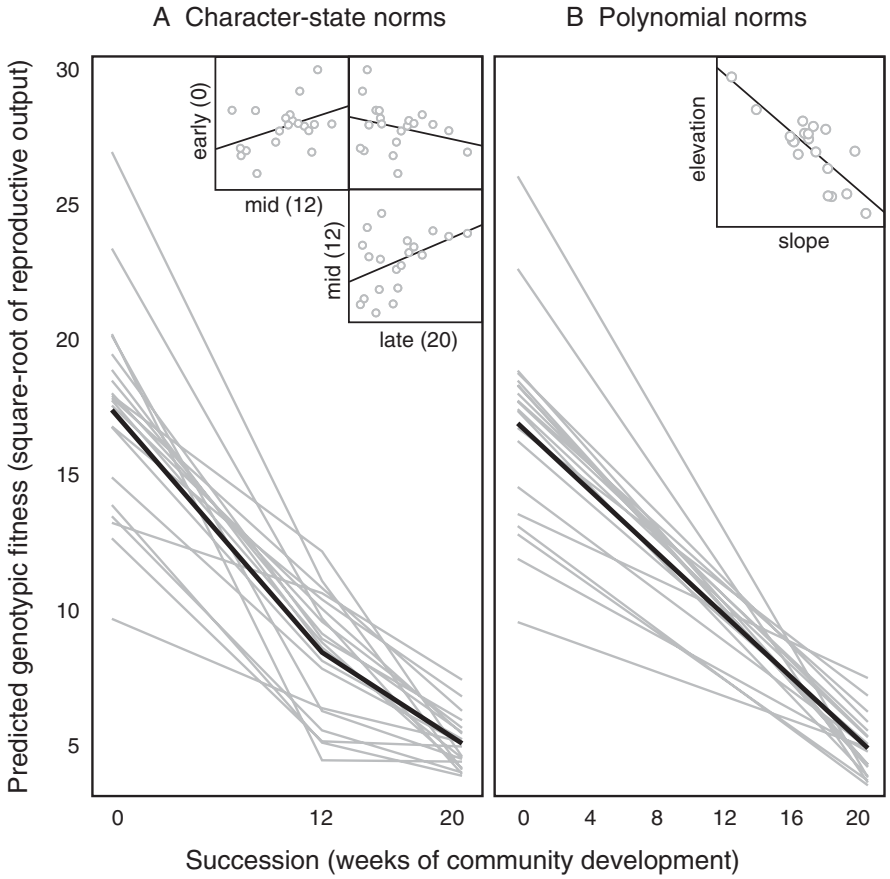

Figure 1. **Reaction norms of *Hippopodina* across a natural range of succession. Two alternative models are presented: reaction norms in (A) are modeled as character-states expressed at discrete successional stages (early, mid, and late, corresponding to 0, 12, and 20 weeks of community development, respectively); reaction norms in (B) are modeled as polynomial functions (defined by elevation and slope) of the successional range. In each panel, gray lines are the norms of individual genotypes and the black line is the population mean; thus, total genetic variation among norms is described by the deviations of gray lines from black, whereas genetic correlations between character-states or polynomial coefficients are described by the inset plots of predicted genotypic values.**

THE MULTIVARIATE STRUCTURE OF HERITABLE VARIATION IN REACTION NORMS

Factor-analytic modeling of the genetic covariance matrix for character-state norms found it to be singular, giving statistical support for only two dimensions of a possible three. These supported dimensions, represented by PC eigenvalues and loadings in Table 2A and visualized in Fig. 2A (see also Table S1 for the reduced-rank matrix from which PCs were extracted) are the multivariate directions in which heritable fitness variation exists and our population can thus evolve. Neither direction available to character-state norms leads to fitness being maximized at all successional stages simultaneously: over 75% of heritable variation in our sample, explained by PC1, predicts higher mean fitness at early- and mid-stages to evolve at the cost of fitness later on. The rest, explained by PC2, predicts higher mean fitness at latter stages to evolve at the cost of fitness earlier on. Overall, this result offers more convincing evidence that reaction norms of *Hippopodina* are evolutionarily constrained across succession, and has the advantage over our earlier tests of character-states by taking into account all their variances and covariances simultaneously.

Factor-analytic modeling of polynomial norms supported a full-rank matrix, with heritable fitness variation present in both possible dimensions (Table 2B). The vast majority (89%) of variation, explained by PC1, predicts slope and elevation to evolve antagonistically (as per their negative correlation in Table 1B), with tolerance of succession improving at the cost of mean fitness over that range, and *vice versa* (Fig. 2B). This result mirrors the structure of heritable variation in character-state norms, although evolving broader tolerance of succession should see mean fitness increase at latter stages but decrease at earlier ones, whereas evolving higher mean fitness throughout succession as a whole should produce the opposite pattern. Unlike character-state norms, a small amount of heritable variation in polynomial norms escapes such constraint, with the imperfect negative correlation between elevation and slope leaving 11% (PC2) free for tolerance and mean fitness to improve jointly (Table 2B and Fig. 2B).

Table 1. Heritable variation in *Hippopodina* reaction norms (described by the deviations of gray lines from black ones in Fig. 1). In (A), norms are modeled as character-states expressed at discrete successional stages; in (B) norms are modeled as polynomial functions of succession.

	Genetic variance component	SE	$LRT_{(1)}$ vs. 0	$LRT_{(1)}$ vs. 1
(A) Character-state norms				
Early succession	0.41	0.16	33.35^{***}	
Mid succession	0.16	0.07	16.01^{***}	
Late succession	0.04	0.02	5.82^{**}	
$Correlation_{(Early, Mid)}$	0.37	0.26	1.66	14.30^{***}
$Correlation_{(Early, Late)}$	−0.17	0.34	0.26	3.23^{*}
$Correlation_{(Mid, Late)}$	0.40	0.31	1.29	3.99^{*}
(B) Polynomial norms				
Elevation	0.08	0.04	18.67^{***}	
Slope	0.11	0.04	34.46^{***}	
$Correlation_{(Slope, Elevation)}$	−0.76	0.14	10.03^{**}	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Discussion

The life-history constraints assumed to support successional diversity have long been sought at the species-level with mixed success (Pacala and Rees 1998; Amarasekare 2003; Clark 2010), leading to renewed focus on the role of individual variation in niche evolution and how variation at this level is maintained (Holt 2009; Araújo et al. 2011). By replicating genotypes of *Hippopodina* across successional stages and taking a holistic approach to analyzing their reaction norms, we show that such variation may reflect an evolutionary constraint on a population's potential to maximize fitness throughout its successional range. In character-state norms, we detected joint patterns of genetic correlation underlying the expression of fitness at different stages, suggesting that adaptation to one successional niche may compromise fitness in others. In polynomial norms, we detected a genetic correlation between mean fitness and the responsiveness

Table 2. The multivariate structure of heritable variation in *Hippopodina* reaction norms. Genetic parameters from each model in Table 1 are presented as the equivalent covariance matrix (G), with variances on the diagonal and covariances (replacing correlations) below it. To the right are the significant dimensions (PC1 and PC2) of each matrix, obtained by factor-analytic modeling within the hypothesis-testing framework of the main model (see Table S1 for the reduced-rank matrix from which PCs were extracted). For each PC, loadings in bold describe the contribution of each parameter to that dimension, whereas loadings in brackets are scaled to describe the correlation of each parameter with that dimension. Eigenvalues describe the amount of variance in G that each PC explains.

	Genetic covariance matrix (**G**)			Supported dimensions of **G**			
	Early	Mid	Late	PC1		PC2	
(A) Character-state norms							
Early succession	0.40			**0.96**	(0.99)	**−0.26**	(−0.15)
Mid succession	0.09[1]	0.16		**0.28**	(0.48)	**0.93**	(0.88)
Late succession	−0.02[2]	0.03[3]	0.04	**−0.09**	(−0.52)	**0.25**	(0.85)
Eigenvalue				0.41		0.13	
% of **G** explained				76%		24%	
	Elevation		Slope	PC1		PC2	
(B) Polynomial norms							
Elevation	0.08			**−0.64**	(−0.92)	**0.77**	(0.40)
Slope	−0.08[4]		0.11	**0.77**	(0.96)	**0.64**	(0.28)
Eigenvalue				0.18		0.02	
% of **G** explained				89%		11%	

[1]SE: 0.08.

[2]SE: 0.04.

[3]SE: 0.03.

[4]SE: 0.03 (SEs for variances are as per Table 1).

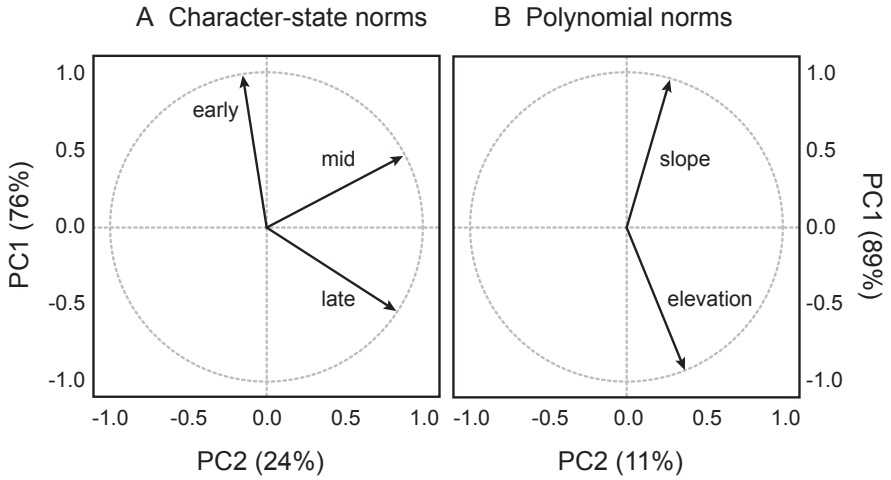

Figure 2. Correlation biplots of the supported dimensions of heritable variation in *Hippopodina* reaction norms, modeled in (A) as character-states expressed at discrete successional stages, and in (B) as polynomial functions of succession. Arrows illustrate the bracketed loadings in Table 2. The angles that arrows form with plot axes describe the genetic correlations between parameters (character-states or polynomial coefficients) and PCs, whereas the angles that arrows form with each other describe the genetic correlations between parameters (angles of 0°, 90°, and 180° indicate correlations of 1, 0, and −1, respectively).

of fitness to succession, suggesting that evolving greater tolerance of this environmental pressure may prove costly overall (with the caveat that a small amount of variation independent of this constraint may permit evolution in a more favorable direction, albeit at a relatively slow rate; Walsh and Blows 2009). Taken as a whole, our results imply that the evolutionary dynamics of *Hippopodina* throughout succession follow the principle that a jack-of-all-trades is master of none, which may help to maintain it as a mix of relatively specialized genotypes occupying distinct successional niches, so long as succession remains spatially structured (e.g., by disturbance) within its dispersal range.

The fact that individuals of every species succumb to adverse environmental conditions led Futuyma and Moreno (1988) to argue that selection has constant opportunity to broaden the species' niche, by expanding either among-individual niche variation (specialization) or within-individual niche width (generalism). Because *Hippopodina* showed limited evolutionary potential to expand the within-individual component of its successional niche in our population, our results imply a constraint on the latter option. Consequently, among-individual niche variation may persist, rather than erode as predicted when populations evolve toward fixation of a generalist genotype (Futuyma and Moreno 1988; Barton 2010). Aspects of this constraint suggest a cost to being a generalist (Lynch and Gabriel 1987; Van Tienderen 1991), with genotypes that tolerated the full successional range with least loss of fitness performing worse, on average, than less-tolerant genotypes that specialized more in its earlier stages. The tendency for early-successional genotypes to be increasingly outperformed by more tolerant genotypes as succession continued, moreover, highlights a useful distinction between fundamental and realized specialization (e.g., Bolnick et al. 2003), with relative generalists seemingly favored to persist as realized specialists in late-successional niches.

Individual specialization is central to Van Valen's (1965) niche variation hypothesis that generalist populations are the products of different individuals being adapted to somewhat different environments. Although criticized for its lack of empirical support (e.g., Grant and Price 1981), Bolnick et al. (2007) argued that past tests of the hypothesis often used poor morphological proxies for niche variation. Analyzing diet instead, they found a population's dietary niche to indeed expand with individual specialization, but noted that their approach could not link specialization of this kind to heredity (as required for its maintenance by selection, and as Van Valen stipulated). Our approach draws on the basics of the hypothesis; namely, that "individuals of some set *a* survive or reproduce better than those of some set *b* in some environment *A*, whereas the reverse is true in some environment *B*" and "the above difference between *a* and *b* is in part genetic" (Van Valen 1965, p. 377). Given the emphasis on fitness in defining the niche, we use the genetic basis of fitness variation throughout succession, evaluated using reaction norms, to infer among-individual niche variation in *Hippopodina*. The advantage of our approach is two-fold: first, it avoids attributing functions to phenotypes that may, in fact, have little bearing on niche variation; second, fitting reaction norms to genotypes provides insight into the heritability of individual specialization that remains surprisingly rare (Araújo et al. 2011). On a cautionary note, there is little consensus on how reaction norms are best modeled, with the character-state and polynomial approaches having different strengths, weaknesses,

and interpretations (see de Jong 1995 or Via et al. 1995 for detailed treatments of this issue). Because neither approach is clearly superior, we present both, and argue that more may be learnt from them together than from either one alone.

The niche variation hypothesis was further undermined by theoretical predictions that niche expansion should occur by increasing within-individual (rather than between-individual) variation, provided that the former is free to evolve (Roughgarden 1972; Taper and Case 1985). This, however, remains an open question: the fact that constraints on such evolution are rarely detected on one hand demands other explanations for specialization (e.g., Fry 1996; Whitlock 1996; Kawecki et al. 1997); alternatively, it could mean that constraints reside in other (or higher-dimensional) phenotypes than are usually tested (Poisot et al. 2011). Treating niche expansion as a special case of reaction-norm evolution (Lynch and Gabriel 1987), we found evidence of constraints in the multivariate structure of heritable variation in reaction norms relating fitness to succession. *Hippopodina* genotypes varied significantly in fitness at each successional stage that we tested, although fitness variation declined up to an order of magnitude as crowding and competition increased, in line with predictions that the expression of variance is suppressed by more stressful conditions (e.g., Hoffmann and Merilä 1999). However, the limited variability of these genotypes in the multivariate direction of highest fitness across the entire successional range may make this a forbidden evolutionary trajectory—that is, little scope to evolve toward it seems to currently exist in our study population (Kirkpatrick 2009; Walsh and Blows 2009). This genetic constraint on reaction-norm evolution is effectively a constraint on the expansion of individual niche width (Lynch and Gabriel 1987; Whitlock 1996). Notably, in the case of character-state norms, it manifested as a higher-dimensional trade-off involving all three measured character-states whose genetic correlations were, in general, quite weak. Upholding the prediction that genetic constraints should become stronger as the number of traits considered increases (Pease and Bull 1988; Walsh and Blows 2009), this result highlights the broader point that multivariate approaches, such as we use here, may often be necessary to uncover such constraints.

Finally, for individual specialization in *Hippopodina* to persist in our study population requires that divergent lineages can stably coexist. The prospect of them doing so will depend on the degree to which they are reproductively isolated, because such lineages may ultimately diverge to the point of speciation without sufficient gene flow among them, or be homogenized by recombination without sufficient isolation (Futuyma and Moreno 1988; Barton 2010). Either way, individual specialization should be lost, leaving the relative spatial scales of gene flow and succession in our system key to its long-term persistence. Because epifaunal communities like ours are prone to frequent, small-scale disturbances, succession will almost certainly vary on scales small enough for extensive gene flow among patches at different stages, as newly-open ones are invaded (via asexual spread, or the dispersal of water-borne gametes and larvae) from undisturbed ones nearby (Connell and Keough 1985). The homogenizing effects of gene flow may be countered, however, by various mechanisms that strengthen isolation, including nonrandom mating within niches and habitat selection (Kawecki 2008; Barton 2010). The scant evidence currently available suggests strong potential for such mechanisms to operate in sessile invertebrate populations, with nonrandom mating reportedly occurring several colonial taxa (Hughes 2005) and a range of marine invertebrate larvae known to avoid settling on substrates occupied by superior spatial competitors (Grosberg 1981). Currently, however, we can only speculate about the relative roles of disturbance, gene flow and such mechanisms of isolation in maintaining individual specialization in *Hippopodina*. Although more insight is certainly needed into the prevalence of multivariate constraints on niche expansion, these issues remain other key frontiers in understanding the evolutionary dynamics of specialization in natural systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Raw reaction norms of *Hippopodina* across a natural range of succession, plotted directly from the data before statistical modeling.

Table S1. The reduced-rank genetic covariance matrix for character-state norms, constrained to be two-dimensional as supported by factor-analytic modeling within the hypothesis-testing framework of the main model (LRT of model fit dropping from full-rank to two dimensions: $\chi^2_1 = 2.70$, $P = 0.10$; LRT of model fit dropping from two to one dimensions: $\chi^2_2 = 12.43$, $P < 0.01$).

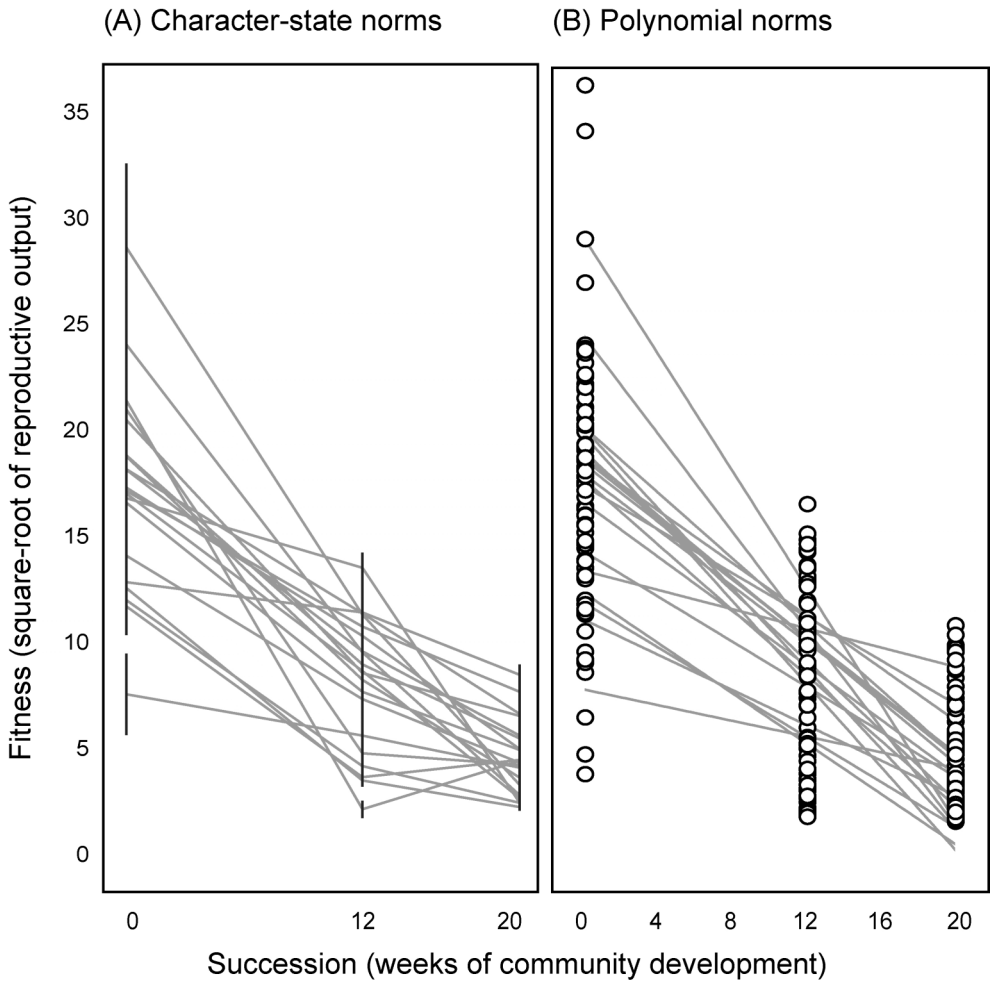

Figure S1. Raw reaction norms of *Hippopodina* across a natural range of succession, plotted directly from the data prior to statistical modelling. Two alternative models are presented: reaction norms in (A) are plotted as character-states expressed at discrete successional stages (early, mid and late, corresponding to 0, 12, and 20 weeks of community development respectively); reaction norms in (B) are plotted as polynomial functions (defined by elevation and slope) of the successional range. In each panel, grey lines are the norms of individual genotypes, combined with all other sources of variation in the experimental design. Error bars around character-state norms are ± 1 SE.

Table S1. The reduced-rank genetic covariance matrix for character-state norms, constrained to be two-dimensional as supported by factor-analytic modelling within the hypothesis-testing framework of the main model (LRT of model fit dropping from full-rank to two dimensions: $\chi^2_1 = 2.70$, $P = 0.10$; LRT of model fit dropping from two to one dimensions: $\chi^2_2 = 12.43$, $P < 0.01$). This is the matrix from which PCs in Table 2 were extracted. No additional matrix is presented for polynomial norms, since factor-analytic modelling supported a full-rank matrix (see Table 2B of the main text) in that case.

	Genetic covariance matrix (**G**)		
	Early	Mid	Late
Early succession	0.385		
Mid succession	0.081	0.147	
Late succession	-0.042	0.020	0.011