

LETTER

Parasites mediate the relationship between host diversity and disturbance frequency

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Abstract

Patterns of community and population diversity are likely to be dependent on interactions between ecological variables. Here we address how two important ecological variables – extrinsic periodic mortality events (disturbances) and the presence of obligate-killing parasites – interact to affect the diversity of niche-specialist genotypes in laboratory populations of the bacterium *Pseudomonas fluorescens*. Consistent with previous studies, diversity was maximized at intermediate frequencies of disturbance in the absence of parasitic bacteriophages (phages). By contrast, no relationship was found between diversity and disturbance frequency in the presence of phage. The results can be explained in part by differential effects of phage on bacterial densities, and hence resource competition, under different disturbance regimes.

Keywords

Bacteria, disturbance, diversity, experimental evolution, parasite, phages.

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INTRODUCTION

Understanding patterns of diversity in natural populations is a major aim of evolutionary biology and ecology. Theoretical and empirical work has identified a number of factors that may affect diversity, both at population and community levels. These include spatial heterogeneity (Hedrick 1986; Rainey & Travisano 1998; Chesson 2000), the amount of nutrients entering the environment (Grime 1973; Rosenzweig 1995), temporal changes in resource availability caused by extrinsic mortality events (disturbances) (Levin & Paine 1974; Connell 1978; Petraitis *et al.* 1989; Chesson 2000) and consumers (parasites, predators, herbivores and parasitoids) (Abrams 2000; Chesson 2000). However, recent work suggests that the effect of an ecological variable on diversity is likely to be contingent on other ecological variables (Huston 1994; Proulx & Mazumder 1998; Kondoh 2001; Worm *et al.* 2002; Orland 2003; Kneitel & Chase 2004). Here we address how obligate-killing parasites interact with disturbances to affect the diversification into spatial niche-specialist genotypes of experimental populations of the bacterium *Pseudomonas fluorescens*.

Theory suggests that intermediate frequencies (and intensities) of disturbance can either directly or indirectly promote coexistence of competitors, hence maximizing diversity. Mechanisms that involve disturbance directly promoting coexistence include the original formulation of

the ‘intermediate disturbance hypothesis’ (Connell 1978), which considered disturbances affecting different patches within a metapopulation at different times. More recently, ‘global’ disturbances, affecting all patches simultaneously, have also been theoretically shown to directly promote coexistence through ‘storage effects’ and ‘relative nonlinearity of competition’ (Chesson & Huntly 1997; Chesson 2000; Roxburgh *et al.* 2004). All these mechanisms require both fitness tradeoffs between pre- and post-disturbance conditions, such that intermediate disturbance favours coexistence of both disturbance tolerant and competitive species, and intraspecific competition to be greater than interspecific competition, such that species will have an advantage when rare (negative frequency-dependent selection). Disturbance can indirectly promote species coexistence simply by reducing mean fitness differences between species adapted to different spatial niches (Chesson & Huntly 1997; Buckling *et al.* 2000; Chesson 2000). It is this latter mechanism that is of relevance to the present study.

A large body of work supports the prediction that species diversity is maximized at intermediate disturbance frequencies (Connell 1978; Sousa 1979; Weider 1992; Sommer 1995; Floder & Sommer 1999; Buckling *et al.* 2000). However, this finding is by no means ubiquitous, with studies reporting no, positive and negative relationships between diversity and disturbance frequency (Petraitis *et al.* 1989; Kocher & Williams 2000; Lake 2000; Mackey & Currie 2001; Ikeda

2003). There are three possible explanations for this. First, with correlational studies of natural populations, other environmental factors that affect diversity may covary with disturbance. Second, some studies may only detect the increasing or decreasing phase of a unimodal diversity–disturbance relationship. Third, the effect of disturbance on diversity may be contingent on other ecological variables, such as parasites and predators.

Studies addressing the effect of inter-trophic interactions on the relationship between species diversity and disturbance are surprisingly limited. One theoretical study (Wootton 1998) found that the unimodal relationship between diversity and disturbance can occur in the presence of a predator, but it may be less likely because the predator also has to promote coexistence of competitors. Data from natural tidal communities also suggests an interaction between disturbance and predation on species diversity, whereby the effect of predation was much more pronounced in areas less disturbed by wave action (Lubchenco & Menge 1978).

In this study, we independently manipulated the frequency of disturbance (removal of a large random proportion of the population) and the presence or absence of a parasite (an obligate-killing virus, phage, that invades and replicates within bacterial cells), to determine if these variables interact in their effect on the diversification of experimental populations of *P. fluorescens*. In spatially heterogeneous environments (static tubes filled with nutrient-rich media), clonal populations of *P. fluorescens* rapidly diversify into spatial niche specialists with distinct colony morphologies (Rainey & Travisano 1998). Ancestral-like smooth (SM) colony types inhabit the broth phase, wrinkly spreader (WS) colony types form a mat at the air–broth interface and, less frequent, fuzzy-spreaders (FS) inhabit the bottom of the tube. Note that considerable variation exists within each of these broad categories, and because reproduction is entirely asexual, each variant is analogous to a species.

Our previous work suggests that phages may increase *P. fluorescens* diversity under disturbance regimes that minimize diversity (high and low disturbance frequencies), but decrease diversity under disturbance regimes that maximize diversity (intermediate disturbance frequencies). Phages increased diversity in spatially homogeneous environments (where little diversification occurs because of a lack of spatial niches) (Brockhurst *et al.* 2004), because of a tradeoff between phage-resistance and competitive ability. This result is consistent with general theory (Holt 1977; Abrams 2000; Chesson 2000; Doebeli & Dieckmann 2000; Abrams 2002; Chase *et al.* 2002), and experimental data on other bacteria–phage interactions (reviewed in Bohannan & Lenski 2000). By contrast, phages decreased diversity in spatially heterogeneous environments (where diversification

into multiple spatial niche specialists can occur), by reducing bacterial density, and hence resource competition-mediated diversifying selection (Buckling & Rainey 2002a; Brockhurst *et al.* 2004).

MATERIALS AND METHODS

Culture conditions

Bacteria were cultured at 28 °C in 30 mL static glass universals with loose plastic lids, containing 6 mL of standard King's medium B (KB). Sixty cultures were initiated with $c. 10^7$ cells of isogenic *Pseudomonas fluorescens* strain SBW25 (Rainey & Bailey 1996), grown for 18 h in KB at 28 °C, shaken at 200 rpm. Thirty tubes were simultaneously inoculated with 10^5 clonal particles of a naturally associated DNA phage, SBW2502 (Buckling & Rainey 2002b). Disturbances were created by transferring 60 µL of culture to fresh medium, representing a 99% mortality rate at each transfer. Six populations with and without phages were transferred either daily, every 2, 4 or 8 days, or not at all, over a 16-day period: $\log_2(\text{disturbance frequencies} + 1)$ of 4, 3, 2, 1 and 0, respectively. Cultures were then frozen in 20% glycerol at –80 °C. Bacterial densities were estimated from colony forming unit counts on KB agar.

Measurement of morphological diversity

Cultures were plated onto KB agar after 16 days, and morphologies of $c. 100$ random colonies visually determined after 48 h incubation at 28 °C (Rainey & Travisano 1998). Within-population diversity was calculated as the complement of Simpson's index of concentration ($1 - \lambda$) (Simpson 1949):

$$1 - \lambda = \left(1 - \sum_i p_i^2 \right) \left(\frac{N}{N-1} \right) \quad (1)$$

where p_i is the proportion of the i th morph and N is the total number of colonies sampled. This measure is the probability that two randomly selected colonies are morphologically different. Final diversity measures for each culture were the average of three replicate platings.

Resistance assays

Resistance of bacterial populations to their co-occurring phage populations was determined by streaking 20 independent bacterial colonies across a line of phage that had previously been streaked on a KB agar plate. Prior to this, phages were isolated from bacteria by centrifuging 900 µL culture with 100 µL of chloroform, which lysed and pelleted bacterial cells, leaving only phages in the supernatant. A

colony was defined as resistant if there was no inhibition of growth, otherwise it was defined as sensitive. Ancestral SBW25 was used on every plate as a control.

Statistical analysis

Diversity was Box–Cox transformed (x^3) to normalize residuals, and analysed as a general linear model, fitting either disturbance frequency or $\log_2(\text{disturbance frequency})$ as both linear and quadratic covariates, the presence or absence of phages as a two-level factor, and all interactions. $\log_{10}(\text{bacterial density})$ was analysed in the same way. To identify specific diversity–disturbance relationships, populations with and without phages were also analysed separately.

To address the mechanisms responsible for different relationships between diversity and disturbance frequency with and without phages, we regressed the change in diversity caused by phage onto (1) the change in density caused by phages and (2) (angular-transformed) proportion resistant bacteria. Changes in diversity were calculated as $[(\text{diversity of individual culture}) - (\text{the mean diversity of phage-free cultures under the same disturbance regime})] / (\text{the mean diversity of phage-free cultures under the same disturbance regime})$. Change in density was calculated in the equivalent way. Both explanatory variables were measured with error, suggesting that model II regression may be appropriate (Sokal & Rohlf 1995). However, model II regressions assume that all error is the result of measurement error. If error is in fact also the result of omission of other explanatory variables, which is likely to be the case here, OLS regression is more appropriate (Grafen & Hails 2002). Analyses were carried out in GENSTAT.

RESULTS AND DISCUSSION

In this study, we addressed how parasites (phage) mediate the relationship between the diversity of niche specialist genotypes of the bacterium *P. fluorescens* and disturbance frequency. We found that the relationship between diversity and disturbance frequency differed when phages were present or absent (Fig. 1. Interaction between $\log_2(\text{disturbance frequency})$ and phage; linear: $F_{1,54} = 10.79$, $P = 0.002$; quadratic: $F_{1,54} = 8.2$, $P = 0.006$). (Note that in this and all subsequent analyses, the same qualitative results were obtained using actual disturbance frequency as the covariate.) Consistent with previous work, diversity showed a unimodal relationship with disturbance frequency in the absence of phages (Fig. 1; quadratic disturbance frequency: $F_{1,27} = 25.65$, $P < 0.0001$): diversity was maximized at intermediate frequencies of disturbance. By contrast, there was no significant relationship between diversity and disturbance frequency in the presence of phages (Fig. 1: $P > 0.2$ for linear and quadratic disturbance

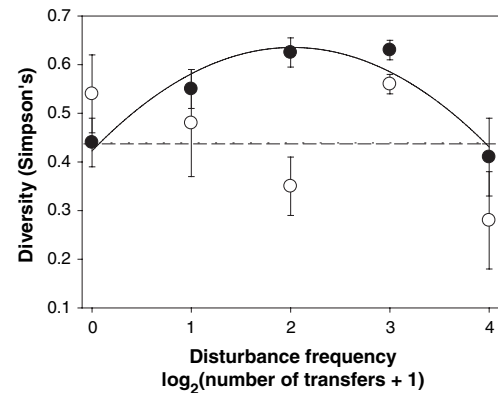


Figure 1 Diversity and disturbance frequency in the presence and absence of phage. Mean (\pm SEM) diversity as a function of disturbance frequency, in the absence (filled circles, solid dashed line) and presence (open circles, dashed line) of phage. Lines are best-fit OLS regression lines.

frequency). This lack of relationship was the result of phages reducing diversity under the second disturbance frequency level [$\log_2(\text{disturbance frequency} + 1)$] (Fig. 1; two-sample t -test with unequal variance: $t = 6.0$, $P < 0.001$, d.f. = 7), but not affecting diversity at other disturbance frequencies ($P = 0.06$ for third disturbance frequency level; $P > 0.1$ for all other disturbance frequencies). Note that variation in diversity is largely the result of variation in evenness of genotypes, rather than variation in number (richness) of genotypes.

Our previous work with this system suggests that maximal diversity under intermediate disturbance frequency (in the absence of phages) was the result of fitness of different spatial niche-specialist genotypes being more equal (Buckling *et al.* 2000), rather than the coexistence of genotypes that are specialized to either high or low disturbance conditions. This was concluded from the fact that the unimodal relationship only arose when there were spatial niches (in static, but not shaken, tubes), and that competition experiments under different disturbance regimes did not reveal a tradeoff between fitness under high and low disturbance frequencies (Buckling *et al.* 2000). Although these tests were not repeated here, the current data is consistent with our previous work: fitness tradeoffs between pre- and post-disturbance conditions associated with the major morph types (SM and WS) did not appear to contribute to the greater diversity at intermediate disturbance frequencies. Despite a tendency for SM morphs to dominate at the lowest disturbance frequency (disturbance frequency of zero; mean proportions \pm SEM: SM = 0.84 ± 0.04 ; WS = 0.16 ± 0.04) and WS morphs to dominate under the highest disturbance frequency (disturbance frequency of 4; SM = 0.16 ± 0.07 ; WS = 0.84 ± 0.07), the intermediate disturbance frequency (disturbance frequency

of 2) did not show more equal numbers of the two major groups of niche specialists ($SM = 0.18 \pm 0.05$; $WS = 0.81 \pm 0.05$). Note that the higher diversity under intermediate disturbance was because of greater evenness within, rather than between, the principal morph types (SM, WS and FS).

We suggest two, not mutually exclusive, reasons why the presence of phages changed the relationship between diversity and disturbance frequency. First, phages differentially affected bacterial density under different disturbance regimes. Previous work has established that phages reduce *P. fluorescens* diversity as a result of reducing bacterial density, which alleviates the strength of resource competition-mediated diversifying selection (Buckling & Rainey 2002a). Phage-mediated reductions in bacterial density are likely to have been greatest when the probability of phage infection was highest (Hochberg & van Baalen 1998); under the disturbance regimes that maximized bacterial density in the absence of phages. Under this hypothesis, we therefore predicted that bacterial density would be greatest, and hence most affected by phages, at intermediate disturbance frequencies. Second, phage-mediated selection of resistant bacterial genotypes may have differed, or contributed to patterns of morphological diversity differently, between different disturbance regimes. Parasites can cause an increase in host diversity if there is a tradeoff between parasite resistance and competitive ability (Abrams 2000; Bohannan & Lenski 2000). Such a tradeoff has been demonstrated in this system: FS genotypes are innately resistant, increasing in frequency in the presence of phages, but are on average less competitive than the other genotypes in the absence of phages (Brockhurst *et al.* 2004). We hypothesized that an increase in the frequency of FS was most likely to have increased diversity when diversity was already low: at high and low disturbance frequencies.

Our data support the hypothesis that phage-imposed bacterial density reductions, and hence reductions in bacterial diversity, were greatest at intermediate disturbance frequencies. In both the presence and absence of phages, bacterial density was maximal at intermediate disturbance frequencies (Fig. 2; quadratic disturbance: $F_{1,54} = 81.56$, $P < 0.0001$). This relationship did not differ between treatments and there was no overall difference in density when phages were present or absent ($P > 0.2$, in both cases). However, densities were significantly reduced by phages under disturbance frequencies of 3 (Fig. 2; two-sample *t*-test with unequal variances [note critical value of $P = 0.0125$ with sequential Bonferroni correction (Rice 1989): $t = 3.47$, $P < 0.01$, d.f. = 9] and 2 ($t = 4.28$, $P < 0.01$, d.f. = 7), but not under the other disturbance frequencies ($P > 0.05$). Further support for the hypothesis that phage-mediated changes in diversity are a positive function of phage-mediated changes in bacterial diversity

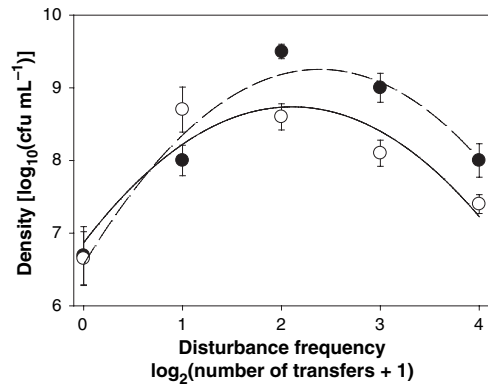


Figure 2 Density and disturbance frequency in the presence and absence of phage. Mean (\pm SEM) \log_{10} [colony forming units (cfu) mL^{-1}] as a function of disturbance frequency, in the absence (filled circles, dashed line) and presence (open circles, solid line) of phage. Lines are best-fit OLS regression lines.

(relative to phage-free populations, under the same disturbance regime) was obtained from the significant positive slope of the regression of change in diversity on change in density ($F_{1,28} = 7.94$, $P < 0.01$, adjusted $R^2 = 19.2\%$).

Tradeoffs between phage resistance and competitive ability (Abrams 2000; Bohannan & Lenski 2000; Chesson 2000; Brockhurst *et al.* 2004) did contribute to changing the composition of the populations. The average proportion of FS genotypes was much greater in cultures with phages than without (9.9% compared with 0.2%; Mann–Whitney test: $P < 0.01$). However, exclusion of FS from the diversity analyses did not qualitatively change the results: there were no significant linear or quadratic relationships between diversity and disturbance frequency in populations with phages ($P > 0.2$, in all cases). This suggests that phage-mediated selection for resistant FS had little impact in changing the diversity–disturbance relationship from unimodal to non-significant. Furthermore, there was no evidence that the strength of selection for phage resistance differed significantly between disturbance regimes: there was no difference in the proportion of resistant bacteria (considering all genotypes) between disturbance regimes (mean proportion resistant = 0.26; generalized linear model with binomial errors: $P > 0.05$), and no relationship between changes in diversity (from control populations) caused by phages and proportion of resistant bacteria ($P > 0.1$).

Despite a significant relationship between phage-mediated changes in density and diversity (relative to phage-free populations), only around 20% of the variance was explained. The large amount of unexplained variation is likely to be because of coevolution between bacteria and phages. Bacteria and phages undergo multiple rounds of reciprocal evolution of resistance and infectivity,

respectively, during the time scale of the current experiment (Buckling & Rainey 2002b). Variation between populations in the rates of coevolution and the timing of the appearance of particular resistance and infectivity mutations is likely to have had a major impact on bacterial diversity and density (Buckling & Rainey 2002b; Brockhurst *et al.* 2004).

We have shown a significant interaction between the presence of parasites and abiotic disturbance frequency on the evolution and maintenance of host diversity: the addition of parasitic phages changed the diversity–disturbance relationship from unimodal to non-significant. Our data suggests that this change was in part the result of phages having the greatest relative impact on bacterial density (and hence resource competition and host diversity) when host density was greatest: at intermediate disturbance frequencies. It is unclear, however, whether this result would have been obtained if a different mechanism was responsible for the unimodal relationship between diversity and disturbance frequency in the absence of phages.

The applicability of these results to natural disturbance–consumer interactions (where disturbance promotes coexistence through equalizing fitness of spatial niche specialists) requires maximum host or prey density to occur at intermediate disturbance frequencies. This is possible: intermediate disturbance frequencies may allow population carrying capacities to be reached, without a subsequent decrease in density, as a result of nutrients becoming very scarce. These data may help to explain why the predicted unimodal diversity–disturbance relationship is often not found in studies of natural populations, where the presence of parasites and predators is inevitable (Petraitis *et al.* 1989; Kocher & Williams 2000; Lake 2000; Mackey & Currie 2001; Ikeda 2003). This work further emphasizes that caution must be taken in interpreting the results of ecological manipulations unless all covarying factors have been controlled for.

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REFERENCES

- Abrams, P.A. (2000). Character shifts of prey species that share predators. *Am. Nat.*, 156(Suppl.), S46–S61.
- Abrams, P.A. (2002). The evolution of traits affecting resource acquisition and predator vulnerability: character displacement under real and apparent competition. *Am. Nat.*, 160, 692–704.
- Bohannan, B.J.M. & Lenski, R.E. (2000). Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.*, 3, 362–377.
- Brockhurst, M.A., Rainey, P.B. & Buckling, A. (2004). The effect of parasites and spatial heterogeneity on the evolution of host diversity. *Proc. R. Soc. Lond. B*, 271, 107–111.
- Buckling, A. & Rainey, P.B. (2002a). The role of parasites in sympatric and allopatric diversification. *Nature*, 420, 496–499.
- Buckling, A. & Rainey, P.B. (2002b). Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. Lond. B*, 269, 931–936.
- Buckling, A., Kassen, R., Bell, G. & Rainey, P.B. (2000). Disturbance and diversity in experimental microcosms. *Nature*, 408, 961–964.
- Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D. *et al.* (2002). The interaction between predation and competition: a review and synthesis. *Ecol. Lett.*, 5, 302–315.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Ann. Rev. Ecol. Syst.*, 31, 343–366.
- Chesson, P. & Huntly, N. (1997). The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.*, 150, 519–553.
- Connell, J.H. (1978). Diversity in tropical rain forests and coral reefs. *Science*, 199, 1302–1310.
- Doebeli, M. & Dieckmann, U. (2000). Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *Am. Nat.*, 156(Suppl.), S77–S101.
- Flöder, S. & Sommer, U. (1999). Diversity in planktonic communities: an experimental test of the intermediate disturbance hypothesis. *Limnol. Oceanogr.*, 44, 1114–1119.
- Grafen, A. & Hails, R. (2002). *Modern Statistics for the Life Sciences*. Oxford University Press, Oxford.
- Grime, J.P. (1973). Control of species diversity in herbaceous vegetation. *J. Environ. Mgmt.*, 1, 151–167.
- Hedrick, P.W. (1986). Genetic polymorphism in heterogeneous environments: a decade later. *Ann. Rev. Ecol. Syst.*, 17, 535–566.
- Hochberg, M.E. & van Baalen, M. (1998). Antagonistic coevolution over productivity gradients. *Am. Nat.*, 152, 620–634.
- Holt, R.D. (1977). Predation, apparent competition, and the structure of prey communities. *Theor. Pop. Biol.*, 12, 197–229.
- Huston, M.A. (1994). *Biological Diversity*. Cambridge University Press, Cambridge.
- Ikeda, H. (2003). Testing the intermediate disturbance hypothesis on species diversity in herbaceous plant communities along a human trampling gradient using a 4-year experiment in an old-field. *Ecol. Res.*, 18, 185–197.
- Kneitel, J.M. & Chase, J.M. (2004). Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecol. Lett.*, 7, 69–80.
- Kocher, S.D. & Williams, E.H. (2000). The diversity and abundance of North American butterflies vary with habitat disturbance and geography. *J. Biogeography*, 27, 785–794.
- Kondoh, M. (2001). Unifying the relationships of species richness to productivity and disturbance. *Proc. R. Soc. Lond. B*, 268, 269–271.
- Lake, P.S. (2000). Disturbance, patchiness, and diversity in streams. *J. North American Bent. Soc.*, 19, 573–592.
- Levin, S.A. & Paine, R.T. (1974). Disturbance, patch formation, and community structure. *Proc. Natl. Acad. Sci. USA*, 71, 2744–2747.

- Lubchenco, J. & Menge, B.A. (1978). Community development and persistence in a low rocky intertidal zone. *Ecol. Monogr.*, 59, 67–94.
- Mackey, R.L. & Currie, D.J. (2001). The diversity–disturbance relationship: Is it generally strong and peaked? *Ecology*, 82, 3479–3492.
- Orland, M.C. (2003). Scale-dependent interactions between intrinsic and extrinsic processes reduce variability in protist populations. *Ecol. Lett.*, 6, 716–720.
- Petraitis, P.S., Latham, R.E. & Niesenbaum, R.A. (1989). The maintenance of species diversity by disturbance. *Q. Rev. Biol.*, 64, 393–418.
- Proulx, M. & Mazumder, A. (1998). Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient-rich ecosystems. *Ecology*, 79, 2581–2592.
- Rainey, P.B. & Bailey, M.J. (1996). Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. *Mol. Microbiol.*, 19, 521–533.
- Rainey, P.B. & Travisano, M. (1998). Adaptive radiation in a heterogeneous environment. *Nature*, 394, 69–72.
- Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, 43, 223–225.
- Rosenzweig, M.L. (1995). *Species Diversity in Space and Time*. Cambridge University Press, Cambridge.
- Roxburgh, S.H., Shea, K. & Wilson, J.B. (2004). The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. *Ecology*, 85, 359–371.
- Simpson, E.H. (1949). Measurement of diversity. *Nature*, 163, 688.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry*. W. H. Freeman and Company, San Francisco, CA.
- Sommer, U. (1995). An experimental test of the intermediate disturbance hypothesis using cultures of marine phytoplankton. *Limnol. Oceanogr.*, 40, 1271–1277.
- Sousa, W.P. (1979). Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. *Ecology*, 60, 1225–1239.
- Weider, L.J. (1992). Disturbance, competition and the maintenance of clonal diversity in *Daphnia pulex*. *J. Evol. Biol.*, 5, 505–522.
- Wootton, J.T. (1998). Effects of disturbance on species diversity: a multitrophic perspective. *Am. Nat.*, 152, 803–825.
- Worm, B., Lotze, H.K., Hillebrand, H. & Sommer, U. (2002). Consumer versus resource control of species diversity and ecosystem functioning. *Nature*, 417, 848–851.

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