

## Testing for local species saturation with nonindependent regional species pools

Jeremy W. Fox, Jill McGrady-  
Steed and Owen L. Petchey  
Department of Ecology,  
Evolution, and Natural  
Resources, Cook College,  
Rutgers University, 14 College  
Farm Road, New Brunswick, NJ  
08901–8551, USA.  
E-mail: jwfox@rci.rutgers.edu

### Abstract

Identifying the factors controlling local community structure is a central problem in ecology. Ecologists frequently use regression to test for a nonlinear saturating relationship between local community richness and regional species pool richness, suggesting that species interactions limit the number of locally coexisting species. However, communities in different regions are not independent if regions share species. We present a Monte Carlo test for whether an observed local-regional richness relationship is significantly different from that expected when regions are nonindependent and species interactions do not limit community membership. We illustrate this test with data from experimental microcosm communities. A conventional *F*-test suggests a significant saturating relationship between realized community richness and species pool richness. However, the Monte Carlo test fails to reject the null hypothesis that species interactions do not affect community richness. Strong species interactions do not necessarily set an absolute upper limit to the number of locally coexisting species.

### Keywords

Community assembly, invasibility, local *versus* regional species richness, microcosms, Monte Carlo test, protists, species interactions, species saturation.

*Ecology Letters* (2000) 3: 198–206

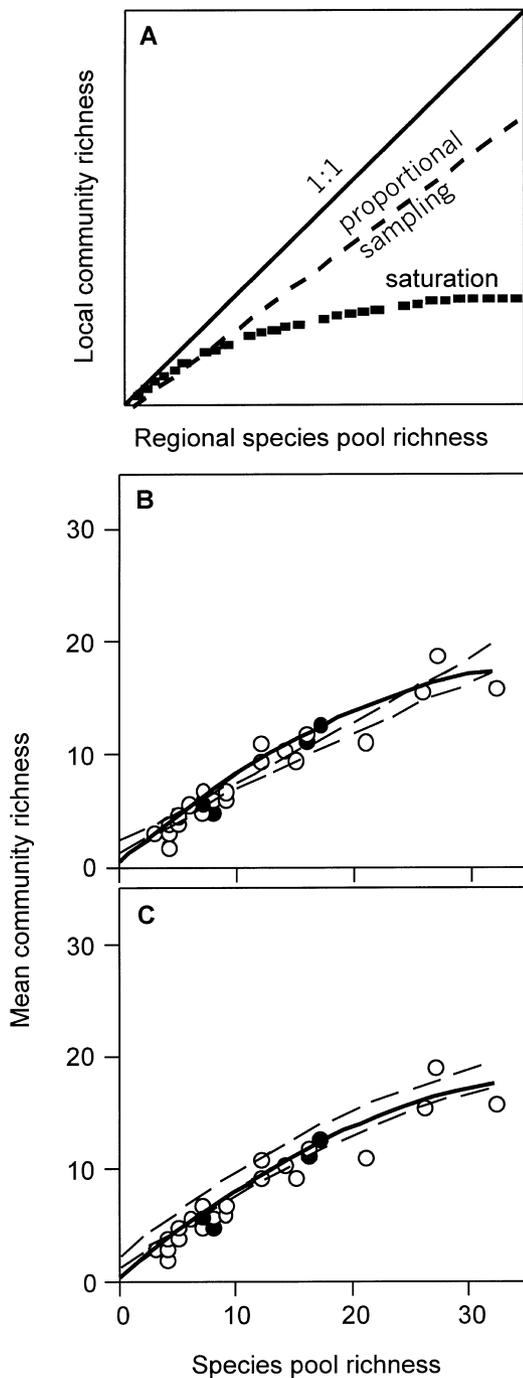
### INTRODUCTION

The control of diversity in local communities is a fundamental question in ecology (Morin 1999). Classical theory emphasizes the role of within-community processes such as competition and predation in limiting community membership to those species that occupy sufficiently dissimilar niches (Levin 1970; MacArthur 1972; Jeffries & Lawton 1984). More recent theory emphasizes the role of large-scale processes external to the community (e.g. speciation, long distance migration), so that community richness reflects the diversity of a regional pool of potential colonists (Ricklefs 1987; Cornell & Lawton 1992; Caswell & Cohen 1993; Huston 1999; Srivastava 1999). More recent theory also emphasizes that strong species interactions need not limit community membership (Cornell & Lawton 1992; Caswell & Cohen 1993; Grover 1994; Loreau 2000).

Plots of the species richness of local communities *versus* the richness of the regional species pools from which the communities were assembled contain information about the factors controlling local species richness (Cornell & Lawton 1992; but see Loreau 2000). A plot of local *versus*

species pool richness should show a concave-down (saturating) curve if local interactions limit community membership (Fig. 1A). After the regional pool contains enough species to fill the available niches, local richness becomes independent of pool richness (Cornell & Lawton 1992). In contrast, a linear relationship indicates that communities simply contain a constant fraction of the regional species pool (proportional sampling (Cornell & Lawton 1992)), either because interspecific interactions are weak or because other factors (e.g. spatial structure, disturbance, invasion sequence) prevent exclusion (Cornell & Lawton 1992; Caswell & Cohen 1993; Grover 1994; see also Loreau 2000).

Comparative studies plotting local richness in similar habitats from different regions against regional richness typically find linear relationships (Srivastava 1999). However, comparative studies have limitations that complicate determination of the form and causes of the local-regional richness relationship (Westoby 1998; Huston 1999; Srivastava 1999; Griffiths 1999). Srivastava (1999) argues that the richnesses of local communities assembled from the same regional species pool are not independent, as assumed in many published analyses,



because regional richness is confounded with species pool composition and geographical location (pseudoreplication, Hurlbert 1984). Here we point out that communities in different regions are also nonindependent when regional species pools overlap in species composition. Nonindependent regional species pools invalidate conventional statistical tests for whether a bivariate plot is better-described by a linear or nonlinear regression.

**Figure 1** Hypothesized (A) and observed (B, C) relationships between species richness of regional species pools and of local communities assembled from those pools. In (A), the 1:1 line represents the limit of equal local and regional richness (redrawn from Cornell & Lawton 1992). In (B, C), filled circles are data from the experiment of Petchey *et al.* (1999); open circles are from McGrady-Steed *et al.* (1997). The regression line (solid line) is given by  $Y = -0.029 + 0.895X - 0.011X^2$ . In (B), dashed lines indicate nonparametric 95% confidence intervals for the estimated value of  $Y$ , based on linear regression (see text for details). In (C), dashed lines indicate nonparametric 95% confidence intervals for the estimated value of  $Y$ , based on quadratic regression (see text for details).

Consider a number of geographical regional species pools of varying richness but harbouring some of the same species. Assume that all species have the opportunity to colonize all local sites within their regions (colonization limitation is a separate problem not considered here) and that regions and sites do not vary except in the richness and composition of regional species pools. If species do not interact strongly enough to limit community membership, each species has a species-specific probability of persisting at a local site in a region. The richness of a local site results from the probabilistic persistence of each species in the regional pool. Richnesses of local sites in different regions are not independent because, to the extent that the regional species pool overlap, richnesses of local sites in different regions are realizations of the same probabilistic processes. Strong species interactions make species persistence probabilities a function of the number and/or identity of the other species in the regional pool, but do not eliminate nonindependence of regional pools with overlapping compositions. In both cases, species pool richness is confounded with species pool composition.

Nonindependence of sites within regions can be eliminated by averaging richness across sites within regions and plotting mean local richness against regional richness (Srivastava 1999). Nonindependence of sites across regions cannot be eliminated this way. Regional species pools frequently overlap (e.g. Terborgh & Faaborg 1980; Cornell 1985a, b; Griffiths 1997; Huguency *et al.* 1997), making correction for nonindependence important for interpreting tests for local species saturation.

Here we describe a Monte Carlo test to account for nonindependence generated by overlapping compositions of regional species pools (Manly 1991). We illustrate this test with data from experimental protist microcosm communities assembled from species pools of varying richness and overlapping composition. Microcosm experiments generate long-term data from real organisms while isolating the influence of nonindependence between

regions by excluding many of the factors complicating interpretation of field data (Westoby 1998; Huston 1999; Srivastava 1999)

## MATERIALS AND METHODS

We assembled replicate aquatic communities in laboratory microcosms from species pools of varying richness and composition (McGrady-Steed *et al.* 1997; Petchey *et al.* 1999; the two studies used similar methods, so we used data from both). Use of experimental species pools allowed accurate, unambiguous determination of species pool richness and composition, realized local richness and local and regional boundaries. Previous papers report the relationship between species richness and ecosystem function in these communities (McGrady-Steed *et al.* 1997; Petchey *et al.* 1999). Here we focus on the relationship between initial (species pool) richness and final (local) richness.

We added bacteria, protists and small metazoans in different species combinations to 250 mL screw-capped Ehrlenmeyer flasks containing 100 mL of nutrient medium (0.56 g of Carolina Biological Supply (Burlington, NC) Protozoan Pellets and 10 g of soil from the Rutgers Display Gardens pond (New Brunswick, NJ) per 3 L of well water) and four wheat seeds. Incubators maintained constant environmental conditions (23°C, 14:10 light:dark cycle (McGrady-Steed *et al.* 1997) or 22°C, 16:8 light:dark cycle (Petchey *et al.* 1999)). Weekly replacement of 7% of the medium and bi-weekly replacement of one wheat seed provided supplemental nutrients.

The experiment involved a total of 39 species (Table 1), used to create species pools which initially contained 0 (bacteria only), 3, 4, 5, 10, 12, 15, 20, 25 or 31 species (some contamination occurred; see below). Each species pool contained species from a variety of trophic groups (primary producers, herbivores, bacterivores and predators). Assignment of many heterotrophs to these trophic groups is somewhat arbitrary because many heterotrophs are quite polyphagous (J. McGrady-Steed, personal observation). We isolated all 39 eukaryotic species used in the experiments from the Rutgers Display Garden Pond (New Brunswick, NJ). This was all the protists and protist-sized metazoans we could isolate, grow in the laboratory and tell apart (based on appearance and behaviour) under a dissecting microscope. The need to tell species apart may have biased our experiments against saturation by ensuring some dissimilarity and niche differences among potential competitors. We assigned species to species pools haphazardly, subject to two constraints. First, to ensure a persistent biodiversity gradient, we excluded from the experiment certain voracious predators (*Didinium nasutum*, *Dileptus* spp.,

*Bursaria* spp.) known to interact strongly with many other species (Gause 1934; Luckinbill 1973; O.L. Petchey, unpublished data). This constraint may have biased our experiments against saturation. Second, each herbivore and predator in a pool could consume at least one other eukaryotic species in the pool. This constraint is biologically realistic (without it, herbivores and predators are guaranteed to starve immediately). We emphasize that the second constraint does not guarantee persistence of herbivores and predators (see, for example, the Results and Weatherby *et al.* 1998). Because of the second constraint and our use of almost all available species in the richest pool, less-rich pools were largely subsets of the richest pool such as might result from species loss across all trophic levels. Other than these two constraints, we made no attempt to ensure that the species in a pool could coexist. The second constraint makes diversity somewhat confounded with species composition and trophic structure (the most depauperate pools lacked predators; Table 1). To minimize confounding, we used several different (but still somewhat overlapping) species combinations to create alternative pools of initially identical richness within the lower portion of the richness gradient (McGrady-Steed *et al.* 1997; Petchey *et al.* 1999; Table 1).

We staggered species introductions by trophic level to ensure abundant prey before predator introduction. We restrict our analysis to eukaryotes because all communities shared the same bacterial species pool. Random assignment of species pools to microcosms ensured that any minor habitat variation was not systematically related to pool richness. We assembled five replicate communities from each initial species pool. All species can grow in microcosms, barring exclusion by interspecific interactions, and consequently no species failed to persist due to lack of suitable habitat. The microcosms are small enough for all species to meet and interact, but large enough to potentially contain large populations which persist for hundreds of generations. We estimated realized local species richness after 6 or 7 weeks (representing dozens to hundreds of eukaryote generations) by counting the number of species in a subsample of sufficient volume to allow reliable detection of populations as sparse as 1–2 individuals mL<sup>-1</sup> (most populations were much denser; McGrady-Steed & Morin 2000). As in other protist microcosm studies, community richness and composition rapidly stabilized, indicating that we were not observing transient properties of community development (McGrady-Steed *et al.* 1997; Petchey *et al.* 1999).

Minor contamination by protists that were not part of the initial species pools occurred in some flasks (McGrady-Steed *et al.* 1997). Most incidents of contamination involved species that were members of other initial species pools (e.g. *Ankistrodesmus* I was an initial member of

**Table 1** Compositions of the species pools (see also McGrady-Steed *et al.* 1997; Petchey *et al.* 1999)

Species	Species pool richness															
	3	4	5	6	7	8	9	12	14	15	16	17	21	26	27	32
<i>Ankistrodesmus</i> I (A)	a	a-c	a		a-c	a	a,c	a-c		a	a		a	a	a	a
<i>Ankistrodesmus</i> II (A)							a,b	a								
<i>Chlamydomonas</i> (A)	a		a,b	a	a-c		a	b,c	a	a,b	a	a	a	a	a	a
Diatom sp. I (A)			a		a,c-e	a	b	a,c	a	b	a	a	a			a
Diatom sp. II (A)					e					b		a				
Desmid sp. (A)												a				
<i>Euglena</i> (A)								a		a	a		a	a	a	a
<i>Netrium</i> (A)										a	a		a			a
<i>Phacus</i> (A)								a						a	a	a
<i>Peridiunium</i> (A)								a					a	a	a	a
<i>Scenedesmus</i> (A)		c	b		c,d	a		b,c	a	a	a	a		a	a	a
<i>Staurastrum</i> (A)							a	b,c	a	b			a	a	a	a
<i>Brachionus</i> (H)		b						b		a	a					a
<i>Frontonia</i> I (H)		c						a,c				a	a	a	a	a
<i>Frontonia</i> II (H)										b						
Hypostome sp. (H)				a	b		b		a			a	a	a	a	a
<i>Stentor</i> I (H)							a		a	b		a	a	a	a	
<i>Stylonychia</i> (H)			b		a,c-e	a		b		b			a	a	a	a
<i>Aspidisca</i> (B)													a	a	a	a
large <i>Amoeba</i> I (B)					b,d	a	a,b	b	a							
small <i>Amoeba</i> (B)		b		a	b,d,e	a	a,b	b	a	b		a				
<i>Coleps</i> (B)																a
<i>Colpidium</i> (B)					e		b	a,b	a	a,b	a		a	a	a	a
small <i>Colpoda</i> (B)														a	a	a
large <i>Colpoda</i> (B)														a	a	a
<i>Halteria</i> (B)	b		a		a			c		a	a	a	a	a	a	a
Gastrotrich sp. (B)								a,c		a	a					a
Microflagellates (B)	a,b	a-c	a,b	a	a-e	a	a,b	b,c	a	b		a	a	a	a	a
<i>Monostyla</i> (B)			b		a,c				a			a	a	a	a	a
<i>Paramecium</i> sp. (B)					d	a		a,c		a,b	a			a	a	a
<i>P. bursaria</i> (B/A)				a					a			a	a	a	a	a
<i>Rotaria</i> (B)					b		a,b	b,c	a	a,b	a			a	a	a
<i>Spirostomum</i> (B/P)								c		a	a	a		a	a	a
<i>Actinosphaerium</i> (P)				a												
Heliozoa sp. (P)					e		b		a	b				a	a	a
large <i>Amoeba</i> II (P)										b				a	a	a
<i>Oxytricha</i> (P)								a						a	a	a
<i>Stentor</i> II (P)								b		a	a	a	a	a	a	a
<i>Urostyla</i> (P)										a	a		a			a

Capital letter(s) in parentheses following species names identify species trophic group(s): autotrophic producer (A), herbivore (H), bacterivore (B) and predator (P). Lower-case letters a–e indicate the compositions of up to five different species pools containing a given number of species. Species pool richness equals the number of eukaryotic species originally composing the pool, plus any species contaminating the pool. Pools 6, 7e, 15b and 17 comprised the constant-temperature treatment of Petchey *et al.* (1999); other species pools are from McGrady-Steed *et al.* (1997).

several species pools, and contaminated the pool that initially contained only bacteria). We defined the actual species pool for each flask as the initial species pool plus any contaminant species observed in any sample from the flask. Different species sometimes contaminated different replicates of the same initial pool, so some actual species pools were replicated less than five times. The final dataset

contained 28 unique species pools of 3–32 species, with 1–5 replicate flasks per pool (Table 1).

We plotted mean realized local richness after 6–7 weeks against actual species pool richness and tested for saturation using second-order polynomial (quadratic) regression. Use of mean local richness avoids pseudo-replication arising from nonindependence within regions

(Griffiths 1999; Srivastava 1999). A negative quadratic coefficient that significantly improves model fit indicates saturation (Cresswell *et al.* 1995). Since we could measure the local-regional richness relationship for very depauperate species pools, we did not force the regression through zero (Cresswell *et al.* 1995; Griffiths 1999; Srivastava 1999).

Nonindependence of regional pools invalidates the conventional *F*-test for whether the additional parameter of a quadratic regression significantly improves model fit over a linear regression (Hurlbert 1984; Sokal & Rohlf 1994). We used a Monte Carlo randomization procedure (Manly 1991) to generate the *F*-distribution expected under the null hypothesis that each species has a constant, species-specific probability of persisting, so that species interactions (however strong) do not affect community membership. If the null hypothesis is true, a species' persistence probability is estimated by the number of jars in which the species persisted divided by the number of jars to which the species was added (or contaminated). We used the estimated persistence probabilities to construct randomized pseudo-data. To determine whether a species persisted in a flask, we randomly drew a 0 (extinction) or 1 (persistence) from a binomial distribution parameterized by that species' observed persistence probability. Repeating this procedure for each species added to (or contaminating) the flask yielded the realized species richness of the flask. Repeating this procedure for every flask yielded a set of pseudo-data, from which we determined the mean realized richness of the pseudo-communities assembled from each pool. A set of pseudo-data had the same 28 species pools, and the same number of replicates assembled from each pool, as the real data. We fit linear and quadratic regressions to the pseudo-data and calculated an *F*-value for the difference in fit. We repeated the entire procedure 1000 times, producing the expected distribution of *F*-values under the null hypothesis of constant, independent species persistence probabilities. The observed *F*-value is significant at the 5% level, suggesting species interactions limit community membership, if it is  $\geq 95\%$  of the Monte Carlo *F*-values. We conducted the randomizations and regressions using MathCad Plus 8.0 Professional Edition for Windows (MathSoft, Inc., Cambridge, MA) running on an IBM PC-compatible computer.

Several species always went extinct, and several always persisted. The randomization procedure treats observed persistence probabilities as estimates of the true probabilities, and the true probabilities are unlikely to equal 0 or 1. We assigned each species that always went extinct a persistence probability of  $1/2n$ , where  $n$  is the number of jars to which that species was added. Analogously, we assigned species that always persisted a persistence probability of  $1-1/2n$ .

We regard the observed persistence probabilities as estimates of species' mean persistence probabilities. An alternative procedure would take the numbers of times each species persisted as fixed. If a species was added to 10 jars and persisted in five of them, that species would persist in five of those 10 jars in each set of pseudo-data. This alternative procedure conditions the analysis on the observed data. We feel our procedure is more appropriate because persistence in this system is somewhat variable, even in communities of initially identical composition (Lawler 1993; Weatherby *et al.* 1998). Our procedure is also a more conservative test for saturation because it introduces more variability into the pseudo-data.

To display the results, we compared our observed quadratic regression line to nonparametric 95% confidence intervals for the estimated value of *Y* (mean realized community richness) at each value of *X* (species pool richness). For any value of *X*, we have 1000 pseudo-estimates of *Y* from linear regressions, and 1000 pseudo-estimates of *Y* from quadratic regressions. We defined the 95% linear regression confidence interval at any value of *X* by the 2.5% and 97.5% percentiles of the distribution of 1000 pseudo-estimates of *Y* from the linear regressions (Manly 1991). We defined the 95% quadratic regression confidence interval at any value of *X* in an analogous way. These confidence intervals facilitate visual comparison of the estimated relationship between local and species pool richness to the average relationship expected under the null hypothesis.

## RESULTS

We found a nonlinear saturating relationship between mean realized community richness and species pool richness (Fig. 1B, C). The observed quadratic regression is not contained by the 95% linear regression confidence interval, suggesting a quadratic regression is a better description of the data (Fig. 1B). The observed quadratic regression falls just inside the 95% quadratic regression confidence interval, suggesting that the observed quadratic regression may be consistent with the quadratic relationship expected under the null hypothesis (Fig. 1C).

Linear and quadratic regressions yielded  $R^2 = 0.909$  and  $R^2 = 0.936$ , giving  $F_{1,25} = 10.547$  in a test for additional explained variance (Sokal & Rohlf 1994). With the conventional *F*-distribution, an *F*-value of 10.547 gives  $P = 0.0013$ , indicating the quadratic term significantly improved model fit. However, species pool nonindependence in this dataset altered the *F*-distribution, biasing a conventional *F*-test toward finding species saturation (Fig. 2). Correcting for pool nonindependence using the distribution of 1000 Monte Carlo *F*-values gives  $P = 0.1050$ , indicating that the null hypothesis of

constant, independent species persistence probabilities cannot be rejected (Fig. 2).

## DISCUSSION

### Statistical issues

Overlapping species pool compositions bias conventional statistical tests of whether a plot of local *versus* regional species richness is better described by a linear or saturating function. The Monte Carlo test suggested here corrects this common statistical problem. Studies of the local-regional richness relationship frequently treat communities assembled from nonindependent species pools as independent (e.g. Terborgh & Faaborg 1980; Cornell 1985a, b; Griffiths 1997; Hugueny *et al.* 1997).

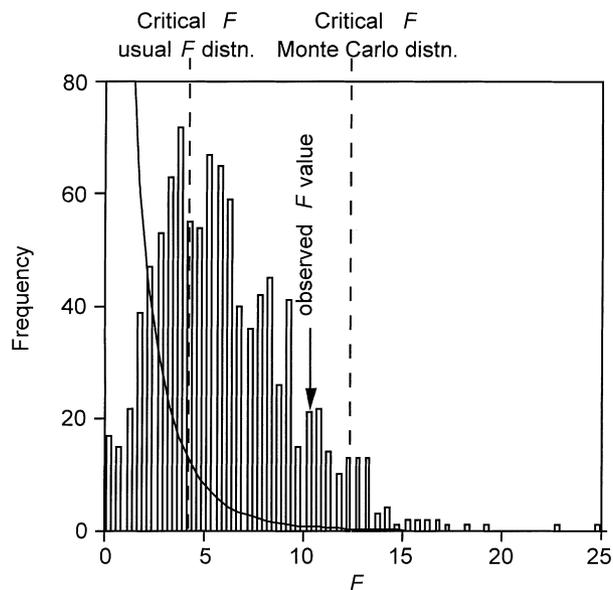
We concur with Gotelli & Graves (1995) that using randomized null models to produce patterns that would be expected in the absence of the ecological factor of interest is a useful approach. Our Monte Carlo procedure tests whether observed data deviate significantly from those expected under a null hypothesis of constant, species-specific persistence probabilities. This null hypothesis is a translation of the ecological null hypothesis that species interactions do not affect community membership into statistical terms. Constructing a null hypothesis that excludes the influence of the factor of

interest while including the influence of other factors is difficult (Gilpin & Diamond 1984; Gotelli & Graves 1995). Our experimental design facilitated construction of this null hypothesis by eliminating a number of factors that would otherwise have had to be modelled as part of the null hypothesis (Huston 1999; Srivastava 1999). Our species pools and local community boundaries were well defined. Communities were physically identical. Species pool richness was defined independently of the composition of local communities. Local communities were large enough to contain viable populations of all species, and were small enough for all species to meet and interact. No species failed to persist due to lack of suitable habitat. We did not have to control for regions of different geographical area. Communities reached compositional equilibrium. A challenge for future comparative studies will be to isolate the effect of species interactions by excluding the influence of confounding factors through the choice of study system and sampling design, or incorporating these factors into the null hypothesis (e.g. Srivastava (1999) shows how to control for variation in region area).

The major drawback of our Monte Carlo test is that observed persistence probabilities may reflect species interactions, and the test may therefore be biased against rejecting the null hypothesis of no species interactions (The Narcissus effect; Colwell & Winkler 1984; Gilpin & Diamond 1984). The observed data appear saturated in part because the richest species pools contained several species that were not members of less-rich pools, and that failed to persist in any jar. The persistence probabilities of these species may be intrinsically small, or merely appear small because the persistence of these species was tested only in challenging, speciose communities. The Monte Carlo test treats these probabilities as intrinsically small, and so fails to reject the null hypothesis of proportional sampling.

Species pool nonindependence biases a conventional  $F$ -test in favour of saturation in our dataset. The overlapping compositions of our species pools makes the expected relationship between local richness and species pool richness slightly curvilinear, even if species do not interact (Fig. 1B, C). Bias in favour of the null hypothesis is also possible (Hurlbert 1984). The direction of bias generated by species pool nonindependence will vary depending on the structure of the data. Whether linear relationships between local and regional richness generally are observed despite, or because, species pool nonindependence is unknown. Simulations exploring the conditions under which species pool nonindependence biases conventional  $F$ -tests for or against the null hypothesis would be useful.

Our Monte Carlo test focuses on correcting for nonindependence of communities in different regions.



**Figure 2** Comparison of the usual  $F$ -distribution (d.f. = 1, 25; solid line) with the distribution of 1000 pseudo- $F$ -values generated by the Monte Carlo procedure (bars). Vertical dashed lines give the critical  $F$ -values ( $P = 0.05$ ) for each distribution. The arrow indicates the observed  $F$ -value for a test of whether quadratic regression (Fig. 1B, C) explains a significantly greater proportion of the variation than a linear regression.

Caley & Schluter (1998) and Srivastava (1999) focus on the nonindependence of communities within the same region. In principle, our Monte Carlo procedure also could be used to correct for nonindependence within regions by omitting the step of averaging realized local richness across sites within regions. This would increase the degrees of freedom for the  $F$ -test for saturation, but also increase the variability of the pseudo-data. This use of the Monte Carlo procedure is most attractive in the (common) cases where the regional species pools are not completely known, and nearby local communities may or may not have been assembled from the same species pool. In these cases, nearby communities are often assumed to share the same regional species pool only because the pools cannot be defined more precisely. Using the Monte Carlo procedure to correct for within-pool nonindependence seems less well motivated when replicate communities are assembled experimentally from known species pools, since standard statistics already provide a way to correct for within-pool nonindependence in this case. The use of randomization tests to correct for within-pool nonindependence deserves further study. Comparisons of the power of within-pool averaging and our randomization test to detect known relationships in simulated data would be especially valuable.

Correcting for the statistical problems created by overlapping species pools is particularly important in light of ecological interpretation problems often created by using nonoverlapping species pools. Hugueny *et al.* (1997) argue that regions with nonoverlapping species pools should not be combined in a single analysis when the regions occupy different continents and have different evolutionary histories. Analysing data from different continents also increases the likelihood of confounding regional richness with physical differences between regions (Srivastava 1999).

### Ecological issues

Our results emphasize that interaction strength and the local-regional richness relationship may be unrelated to one another. Abundant independent evidence indicates strong species interactions and community saturation in our flasks. Strong species interactions resulting in reduced abundances and extinctions are common in protist microcosm studies, although unlike our study, many other studies purposefully choose species known to interact strongly (e.g. Gause 1934; Vandermeer 1969; Lawler 1993; Weatherby *et al.* 1998). In our study, communities assembled from richer species pools exhibited density compensation and resisted invasion by *Euplotes* sp. (McGrady-Steed *et al.* 1997; McGrady-Steed & Morin 2000). Invasibility experiments are the most

direct test of whether species interactions limit community membership. Despite strong independent evidence for saturation, our Monte Carlo test failed to reject the null hypothesis of constant, independent persistence probabilities, and perhaps more importantly, visual inspection of our local-regional richness relationship indicates only a small deviation from linearity (Fig. 1B). Failure to find a strongly concave-down relationship between local and regional richness in this system suggests that such relationships are unlikely to be found in field datasets, even when species interactions are strong. We had many more regional pools (and thus much greater statistical power) than most datasets, and our experimental approach eliminated many potential confounding factors (Huston 1999; Srivastava 1999). Our microcosms also lacked many of the mitigating factors that can prevent species interactions from limiting community membership (e.g. disturbance (Caswell & Cohen 1993), spatial structure (Shorrocks & Rosewell 1986; Armstrong 1989; Holyoak & Lawler 1996; Tilman & Kareiva 1997), invasion sequence (Grover 1994)). Communities of strong interactors may not produce a detectable, strongly concave-down relationship between local and regional richness.

Our results are consistent with recent theory questioning any connection between species interactions and the local-regional richness relationship, but this theory is controversial. Loreau (2000) argues that species interactions only limit local community membership relative to a given species pool. More speciose communities will not necessarily resist novel invaders (i.e. species not part of the original pool) any more than less speciose communities. Although stable coexistence requires niche differences (Chesson & Huntly 1997), local richness will not necessarily reach an upper limit independent of changes in the species pool unless there is an absolute limiting similarity (Abrams 1983; Loreau 2000). Contrary to Loreau (2000), community assembly simulations typically find (or strongly suggest) an absolute upper limit to local richness (Case 1990; Law & Morton 1996; Morton & Law 1997). However, these findings may not be robust (Levine & D'Antonio 1999). All simulation studies to date have assembled resident communities from species pools of varying richness and then tested community invasibility with invaders drawn from the same species pool as residents, or with invader and resident interaction strengths drawn from the same distribution (Levine & D'Antonio 1999). Whether more speciose communities tend to resist truly novel invaders is unknown and deserves further empirical and theoretical study. Testing community invasibility with many different invaders could reveal the relationship between invader success and the traits of resident and invader species (McGrady-Steed *et al.* 1997; Law *et al.* 2000).

We tested for saturation at the whole-community level, while most studies test for saturation only within guilds of similar species (Srivastava 1999). Some authors argue that only species within guilds compete, so combining multiple guilds in a single analysis dilutes the effects of within-guild competition (Gilpin & Diamond 1984). This ignores the potential for predation, and indirect effects other than exploitative competition (e.g. apparent competition, longer-chain indirect effects), to limit community membership (Holt 1977; Yodzis 1988; Menge 1995; Morton & Law 1997). Given the scarcity of data on indirect effects in most systems, the idea that only intraguild competition limits community membership should be treated as a hypothesis rather than an assumption. This hypothesis is difficult to test with our data because many of our heterotrophs are quite polyphagous and assigning them to guilds is somewhat arbitrary.

## CONCLUSIONS

The Monte Carlo test suggested here corrects a widespread statistical difficulty in many published local-regional richness analyses. We urge continued development of null models making quantitative predictions about the expected form of the local-regional richness relationship at different scales in the absence of strong species interactions (Cornell & Lawton 1992; Gotelli & Graves 1995; Huston 1999; Srivastava 1999; Loreau 2000). Because it will always be difficult to statistically separate the effects of species interactions from other effects, independent evidence for (or against) saturation is crucial. Experimental introductions of novel species into communities of varying richness provide the strongest evidence of whether species interactions produce saturation. Understanding the control of diversity remains an important fundamental problem in community ecology.

## ACKNOWLEDGEMENTS

P.J. Morin, H. Stevens, Z. Long, T. Blackburn, H. Cornell, P. Warren, and M. Loreau commented on earlier versions of this work, and P. Smouse provided wisdom on Monte Carlo tests. We thank J. Grover and three anonymous reviewers for helpful criticisms and suggestions, particularly the reviewer who alerted us to regional nonindependence and suggested a randomization test. We thank the National Science Foundation for financial support through grant DEB-9806427 to P.J.M. and T. Casey. J. M.-S. received additional support from NSF grant DEB-9424494 to P.J.M. and T.C. J.W.F. received support from a Rutgers University Hoffman-LaRoche Fellowship.

## REFERENCES

- Abrams, P.A. (1983). The theory of limiting similarity. *Annu. Rev. Ecol. Syst.*, 14, 359–376.
- Armstrong, R.A. (1989). Competition, seed predation, and species coexistence. *J. Theor. Biol.*, 141, 191–194.
- Caley, M.J. & Schluter, D. (1998). The relationship between local and regional diversity: reply. *Ecology*, 79, 1827–1829.
- Case, T.J. (1990). Invasion resistance arises in strongly interacting species-rich model competition communities. *Proc. Natl. Acad. Sci. USA*, 87, 9610–9614.
- Caswell, H. & Cohen, J.E. (1993). Local and regional regulation of species-area relations: a patch-occupancy model. In: *Species Diversity in Ecological Communities* (eds Ricklefs, R.E. & Schluter, D.). University of Chicago Press, Chicago, pp. 99–107.
- Chesson, P. & Huntly, N. (1997). The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.*, 150, 519–553.
- Colwell, R.K. & Winkler, D.W. (1984). A null model for null models. In: *Ecological Communities: Conceptual Issues and the Evidence* (eds Strong, D.R., Simberloff, D., Abele, L.G. & Thistle, A.B.). Princeton University Press, Princeton, pp. 297–315.
- Cornell, H.V. (1985a). Local and regional richness of cynipine gall wasps on Californian oaks. *Ecology*, 66, 1247–1260.
- Cornell, H.V. (1985b). Species assemblages of cynipid gall wasps are not saturated. *Am. Nat.*, 126, 565–569.
- Cornell, H.V. & Lawton, J.H. (1992). Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *J. Anim. Ecol.*, 61, 1–12.
- Cresswell, J.E., Vidal-Martinez, V.M. & Crichton, N.J. (1995). The investigation of saturation in the species richness of communities: some comments on methodology. *Oikos*, 72, 301–304.
- Gause, G.F. (1934). *The Struggle for Existence*. Williams & Wilkins, Baltimore.
- Gilpin, M.E. & Diamond, J.M. (1984). Are species co-occurrences on islands non-random, and are null hypotheses useful in community ecology? In: *Ecological Communities: Conceptual Issues and the Evidence* (eds Strong, D.R., Simberloff, D., Abele, L.G. & Thistle, A.B.). Princeton University Press, Princeton, pp. 297–315.
- Gotelli, N.J. & Graves, G.R. (1995). *Null Models in Ecology*. Smithsonian Institution Press, Washington, D.C.
- Griffiths, D. (1997). Local and regional species richness in North American lacustrine fish. *J. Anim. Ecol.*, 66, 49–56.
- Griffiths, D. (1999). On investigating local-regional richness relationships. *J. Anim. Ecol.*, 68, 1051–1055.
- Grover, J.P. (1994). Assembly rules for communities of nutrient-limited plants and specialist herbivores. *Am. Nat.*, 143, 258–282.
- Holt, R.D. (1977). Predation, apparent competition, and the structure of prey communities. *Theor. Popul. Biol.*, 12, 197–229.
- Hugueny, B., Tito de Morais, L., Mériçoux, S., de Mérona, B. & Ponton, D. (1997). The relationship between local and regional species richness: comparing biotas with different evolutionary histories. *Oikos*, 80, 583–587.
- Hurlbert, S.H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.*, 54, 187–211.
- Holyoak, M. & Lawler, S.P. (1996). Persistence of an extinction-prone predator–prey interaction through metapopulation dynamics. *Ecology*, 77, 1867–1879.

- Huston, M.A. (1999). Local processes and regional patterns: appropriate scales for understanding variation in the diversity of plants and animals. *Oikos*, 86, 393–401.
- Jeffries, M.J. & Lawton, J.H. (1984). Enemy free space and the structure of ecological communities. *Biol. J. Linn. Soc.*, 23, 269–286.
- Law, R. & Morton, R.D. (1996). Permanence and the assembly of ecological communities. *Ecology*, 77, 762–775.
- Law, R., Weatherby, A.J. & Warren, P.H. (2000). On the inviability of persistent protist communities. *Oikos*, 88, 319–326.
- Lawler, S.P. (1993). Direct and indirect effects in microcosm communities of protists. *Oecologia*, 93, 184–190.
- Levin, S.A. (1970). Community equilibria and stability, and an extension of the competitive exclusion principle. *Am. Nat.*, 104, 413–423.
- Levine, J.M. & D'Antonio, C.M. (1999). Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, 87, 15–26.
- Loreau, M. (2000). Are communities saturated? On the relationship between  $\alpha$ ,  $\beta$ , and  $\gamma$  diversity. *Ecol. Lett.*, 3, 73–76.
- Luckinbill, L.S. (1973). Coexistence in laboratory populations of *Paramecium aurelia* and its predator *Didinium nasutum*. *Ecology*, 54, 1320–1327.
- MacArthur, R.A. (1972). *Geographical Ecology*. Princeton University Press, Princeton.
- Manly, B.F.J. (1991). *Randomization, Bootstrap, and Monte Carlo Methods in Biology*, 2nd edn. Chapman & Hall, London.
- McGrady-Steed, J., Harris, P.M. & Morin, P.J. (1997). Biodiversity regulates ecosystem predictability. *Nature*, 390, 162–165.
- McGrady-Steed, J. & Morin, P.J. (2000). Biodiversity, density compensation, and the dynamics of populations and functional groups. *Ecology*, 81, 361–373.
- Menge, B.R. (1995). Indirect effects in marine rocky intertidal interaction webs: patterns and importance. *Ecol. Monogr.*, 65, 21–74.
- Morin, P.J. (1999). *Community Ecology*. Blackwell Science, Malden.
- Morton, R.D. & Law, R. (1997). Regional species pools and the assembly of local ecological communities. *J. Theor. Biol.*, 187, 321–331.
- Petchey, O.L., McPhearson, T., Casey, T.M. & Morin, P.J. (1999). Environmental warming alters food-web structure and ecosystem function. *Nature*, 402, 69–72.
- Ricklefs, R.E. (1987). Community diversity: relative roles of local and regional processes. *Science*, 235, 167–171.
- Shorrocks, B. & Rosewell, J. (1986). Guild size in drosophilids: a simulation model. *J. Anim. Ecol.*, 55, 527–541.
- Sokal, R. & Rohlf, F.J. (1994). *Biometry*, 3rd edn. W.H. Freeman, New York.
- Srivastava, D.S. (1999). Using local-regional richness plots to test for species saturation: pitfalls and potentials. *J. Anim. Ecol.*, 68, 1–16.
- Terborgh, J.W. & Faaborg, J. (1980). Saturation of bird communities in the West Indies. *Am. Nat.*, 116, 178–195.
- Tilman, D. & Kareiva, P., eds. (1997). *Spatial Ecology: the Role of Space in Population Dynamics and Interspecific Interactions*. Princeton University Press, Princeton.
- Vandermeer, J.H. (1969). The competitive structure of communities: an experimental approach with Protozoa. *Ecology*, 50, 362–371.
- Weatherby, A.J., Warren, P.H. & Law, R. (1998). Coexistence and collapse: an experimental investigation of the persistent communities of a protist species pool. *J. Anim. Ecol.*, 67, 554–566.
- Westoby, M. (1998). The relationship between local and regional diversity: comment. *Ecology*, 79, 1825–1827.
- Yodzis, P. (1988). The indeterminacy of ecological interactions as perceived through perturbation experiments. *Ecology*, 69, 508–515.

#### BIOSKETCH

Jeremy W. Fox is interested in community ecology, particularly food webs. His research includes work on exploitative and apparent competition, community-level responses to environmental change and the effect of food web structure on propagation of indirect effects.

Editor, J.P. Grover

Manuscript received 22 November 1999

First decision made 15 December 1999

Manuscript accepted 13 March 2000