BRIEF COMMUNICATIONS ARISING

Competition, predation and natural selection in island lizards

ARISING FROM R. Calsbeek & R. M. Cox *Nature* **465,** 613–616 (2010)

Discerning the relative influence of competition and predation as selective forces is an important goal of evolutionary ecology. Calsbeek and Cox[1] argue that intraspecific competition outweighs predation as an agent of natural selection on island populations of the lizard *Anolis sagrei*. However, we identify several problems with the design and analysis of the Calsbeek and Cox[1] study that we believe render its results uninterpretable.

Calsbeek and Cox[1] manipulated lizard population density and predator occurrence on four small islands. The predation manipulation had three treatments: 'none' (netting covering islands to exclude birds); 'birds' (netting placed around the perimeter of, but not covering, islands, allowing bird access); and 'birds and snakes' (three snakes added to islands without any netting). Lizards were introduced onto islands such that each predation treatment was paired once with a 'high' and once with a 'low' density treatment, although statistical analyses treated density as a continuous variable (contrary to the impression given by their Fig. 2). Over two years (2008 and 2009), each possible combination of the three predation treatments and two lizard-density treatments was established one time (each trial lasting 4 months, with two islands used in both years and two in 2009 only). In addition, one unmanipulated island, Kidd Cay (Fig. 1a), was included in 2008. On each island, the authors recorded survival, habitat use and natural selection on several traits.

This experimental design is confounded in three fundamental ways. First, density is confounded with island area. All analyses treat lizard density as a surrogate for intraspecific competition. However, an inverse correlation with island area explains 95% of the variation in density (Fig. 1b), such that it is impossible to disentangle the two factors statistically. This is a crucial problem, because multiple factors related to both predation and competition are known to vary with island area. For example, as island area increases, so too do the number of bird species[2,3] (which increases the number of potential predators) and mean vegetation height[3] (which might increase lizards' susceptibility to avian predation[4]). Likewise, because larger islands have lower perimeter/area ratios, they receive relatively lower input of marine-resource subsidies and have lower arthropod densities[5]; a study of *A. sagrei* in this system showed that lizard densities vary significantly with the amount of seaweed deposition, and that experimental seaweed deposition increased lizard densities by more than 60% (ref. 6).

Because of these relationships, there is no way to distinguish the relative importance of 'competition' (that is, density) versus predation in driving the results. This point is illustrated by the fact that density and island area are essentially equivalent predictors of both survival (Fig. 1c, d) and year-corrected selection differentials ($r^2 = 0.66$ versus 0.74, respectively, for snout–vent length; 0.50 versus 0.52 for hindlimb length; and 0.92 versus 0.83 for stamina; r^2 values drawn from the better-fitting regression of y against either x or x^{-1}). Thus, we believe that the density–area correlation alone invalidates the conclusion[1] that intraspecific competition drives selection on *A. sagrei*.

There is another problem with the claim[1] that intraspecific competition caused the observed variability in selection differentials. If competition for resources drives natural selection, then greater densities should have negative effects, such as lower survival, on individuals. However, our re-analysis of the data shows that survival is actually

positively correlated with density (Fig. 1c). Thus, the assumption that density is a proxy for competition—which underpins the entire study[1]—seems unwarranted. Indeed, we can think of no plausible causal explanation for a direct positive relationship between density and survival in *A. sagrei*. Instead, we suspect that this relationship is an artefact of the near-perfect correlation between density and island area (Fig. 1b), and that differences in survival are actually driven by indirect island–area effects (Fig. 1d). As discussed above, previous work suggests multiple explanations for an inverse relationship between lizard survival and island area[2–6], any or all of which might have operated in this study.

The second structural flaw in the design is the confounding of treatment with year. The birds-and-snakes treatment was applied only in 2009, which makes it impossible to separate the effects of snake addition from the effects of year. The authors controlled for year effects in their analyses of selection by analysing residuals of the regression of selection differentials against year. But because snake addition was only conducted in one year, removing year effects also partially removes any effects of snake addition.
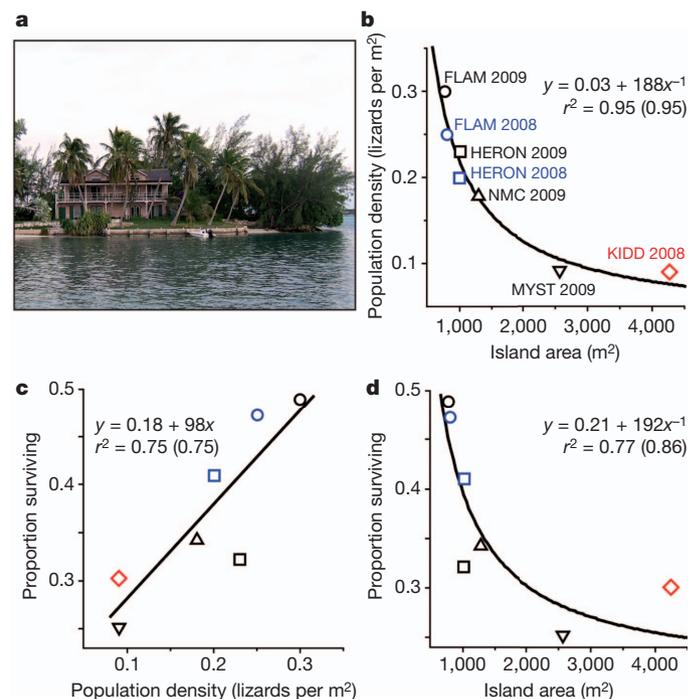
Figure 1 | Confounding relationships among treatments and variables.
a, Kidd Cay, which differs markedly from other islands used in the study (compare with Supplementary Fig. 1a of Calsbeek and Cox[1]). **b**, Strong inverse correlation between lizard density and island area. Islands re-used in successive years share the same symbol (names from Calsbeek and Cox[1]), with those from 2008 shown in blue and Kidd Cay shown in red; r^2 value in brackets is that obtained when Kidd Cay is excluded from the analysis. **c**, Positive correlation between density and survival, probably an artefact stemming from the inverse relationships between density and area (**b**) and survival and area (**d**). All analyses use data presented in or calculated from Supplementary Table 1 of Calsbeek and Cox[1].

Moreover, the correction for year was applied inconsistently. Ordinarily, such a correction would be applied only to variables showing significant variability between years. Instead, Calsbeek and Cox[1] removed year effects in all analyses of selection differentials, even though only one variable differed significantly from 2008 to 2009 (stamina: ANOVA, $F_{1,5} = 26.2$, $P < 0.004$). In contrast, year effects were not removed from analyses of survival, even though male survival was 50% lower in 2009 than in 2008 ($F_{1,4} = 24.3$, $P = 0.007$; also see below). Calsbeek and Cox[1] report that the predation treatments reduced survival, but if year effects had been removed from the survival analyses, they would have found no significant effect of predation on survival. Conversely, the authors report significant effects of population density on selection differentials after correcting for year effects, but none of the selection differentials is significantly related to density when year effects are not removed (generalized linear models with normal distribution and identity link function using JMP 8.02 software, all $P > 0.05$). Thus, the inconsistent handling of year effects determines the main conclusions of the paper, whereas a consistent approach to year effects would have failed to provide support for one or the other set of conclusions.

The third confounding relationship in the design of the Calsbeek and Cox study[1] is between year and sex ratio. The two islands manipulated in 2008 were seeded with 40 males and ~160 females (1:4 sex ratio), whereas the four islands manipulated in 2009 received ~80 males and ~150 females (1:1.9 ratio). Male *A. sagrei* are very aggressive towards conspecific males; therefore, greater male/female ratios might lead to increased agonistic behaviour between males, which is energetically costly and likely to increase predation risk[7]. Thus, the 100% increase in male/female ratio in 2009 might have caused the aforementioned 50% decrease in male survival observed in that year (regression of survival against sex ratio: $r = -0.93$, $F_{1,4} = 25.6$, $P = 0.008$).

Calsbeek and Cox attributed survival differences to predation because survival was lowest on islands with birds and snakes (Fig. 1 of ref. 1). What we show above is that because the two islands with both birds and snakes existed only in 2009, and therefore received twice as many males per female as islands manipulated in 2008, it is impossible to make any causal inference about variation in male survival. The observed variation might have been caused by effects of sex ratio, by environmental differences across the two years of the study, by the predation manipulation, or by some combination or interaction of these three factors.

In addition to these three confounding relationships, a fourth problem in the study design involves the unmanipulated island Kidd Cay, which was "monitored…as a natural reference population". However, Kidd Cay was used as more than just a reference point, because it was included in statistical analyses of selection strength as a 'bird-only' island. (However, it was not included in analyses of lizard survival, which would have eliminated the reported effect of predation on survival[1].) The inclusion of this unmanipulated island in tests of the experimental effects of density and predation is inappropriate because Kidd Cay is qualitatively different from the experimental islands (Fig. 1a): it is much larger, has a hotel on it, is connected by a causeway to an even larger island, and supports domestic predators, *Anolis* species other than *A. sagrei*, and large trees (some ≥10-m tall), none of which occurs on the experimental islands (where most trees are <3-m tall).

Additional concerns include the absence of information necessary to replicate several of the analyses (for example, the final two sentences of the Methods describe analyses not reported in the paper, and no

substantive methods are provided for the analyses in Table 1); statistical non-independence of replicates resulting from the re-use of two islands in successive years; potential biases arising from the use of AIC$_c$ to compare models with 3–5 parameters when $n = 7$; and failure to control for the effect of placing netting on islands in the birds-and-snakes treatment, which confounds the presence of snakes with the absence of netting. Any of these issues might have influenced the results. However, the web of confounding correlations among the variables (especially the statistical near-equivalence of density and island area and the positive density–survival relationship, which together invalidate the use of density as a proxy for competition) means that neither post-hoc statistical palliatives nor the exclusion of Kidd Cay from analyses can resolve the relative importance of competition and predation as agents of selection in this experiment.

The recent advent of experimental field studies in evolution promises investigation of theoretical predictions once thought untestable. In conducting such field studies, however, evolutionary biologists must ensure adequate replication, include appropriate controls for all manipulations, and scrutinize potentially confounding correlations between variables. Ecologists have grappled with these issues for decades, and the ecological literature offers guidance for dealing with them. We sympathize with the difficulties of conducting large-scale field experiments, and we applaud both the vision that motivated this study and the inclusion of the raw data in the Supplementary Information, but unfortunately those data cannot answer the central question posed by Calsbeek and Cox[1]. Thirty years of research in this Bahamian island system suggest that both competition and predation can influence selection, yet we still await a robust experimental test of their relative importance.

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Calsbeek & Cox reply

We agree with several of the points raised by Losos and Pringle[1], but we show here that our data[2] still implicate competition as an agent of natural selection, while providing only limited support for a role of predation. Although patterns of density-dependent survival and selection on Kidd Cay are highly congruent with those on experimental islands, this site could be considered fundamentally different. We therefore base our rebuttal on analyses that use mean values per island (as in our original paper[2]) but which now exclude Kidd Cay ($n = 6$ experimental islands).

We agree that an ideal experimental design would balance predator treatments across years, but we note that the benefits of large-scale experiments often outweigh necessary sacrifices in replication[3,4]. Given that survival was higher in 2008 than in 2009, some of the treatment effects on survival in our Fig. 1 (ref. 2) do reflect year effects. However, during 2009, the year in which all predator treatments were included, survival of males still tended to be lower on islands exposed to bird and snake predators (mean survival = 0.20, 0.30) than on other islands (0.34, 0.35) (generalized linear model (GLM) with identity link function: $\chi^2 = 2.68$, $P = 0.10$; $n = 4$). More importantly, there is no evidence that predators influenced selection on any trait, whether year effects are included (GLM: all $P > 0.26$) or excluded (GLM: all $P > 0.33$). The same is true when analyses are restricted to 2009 (GLM: all $P > 0.21$; $n = 4$). When individual survival (0 or 1) is analysed as the response variable, predator treatment affects overall survival in 2009 (GLM with logit link: $\chi^2 = 47.59$, $P < 0.0001$; $n = 323$), yet no treatment×phenotype interactions are significant (GLM: all $P > 0.43$). This comparison does not provide replication at the population level, but it strongly suggests that predators had little effect on phenotypic selection.

By contrast, population density tends to be associated with phenotypic selection regardless of whether density is treated as a categorical (high/low) variable (GLM all with identity link for snout–vent length: $\chi^2 = 3.15$, $P = 0.08$; hindlimb length: $\chi^2 = 3.32$, $P = 0.07$; stamina: $\chi^2 = 5.01$, $P = 0.03$) or as a continuous variable in analyses including year effects (GLM for snout–vent length: $P = 0.25$; hindlimb length: $P = 0.04$; stamina: $P < 0.001$). Moreover, a two-factor GLM (identity link) with predator and density treatments reveals a significant effect of density, but not predators, for selection on snout–vent length (density: $\chi^2 = 4.51$, $P = 0.03$; predators: $\chi^2 = 2.46$, $P = 0.29$) and stamina (density: $\chi^2 = 6.02$, $P = 0.01$; predators: $\chi^2 = 4.03$, $P = 0.13$). Both density (GLM $\chi^2 = 17.17$, $P < 0.001$) and predators (GLM $\chi^2 = 15.42$, $P < 0.001$) influenced selection on hindlimb length, but predator effects occur because selection was only observed in the absence of predators. Therefore, analyses that exclude Kidd Cay and use only uncorrected selection differentials support the main conclusion of our paper[2] by showing that density influenced selection on each of these traits, whereas predators had little effect on selection.

A more general issue is the extent to which lizard density can be interpreted as a surrogate for competition. We agree that the positive correlation between survival and density (Losos and Pringle[1], Fig. 1c) challenges this assumption. However, the focal result of our study was to show that the relationship between survival and phenotype changed as a function of density, not that overall mortality differed among treatments. Losos and Pringle[1] show that island area is correlated with density and propose the interesting alternative hypothesis that selection could be driven by factors related to island area. We see no reason to consider this a more parsimonious interpretation at present, but we agree that future experiments must explicitly disentangle the effects of density and island area.

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