

1 No conclusive evidence of group selection in spiders

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12 Any field study showing convincing evidence of group selection [GS] would be a significant
13 contribution to the field of evolutionary biology. Pruitt and Goodnight (2014)¹ [PG14] claim to
14 provide such evidence in a 14-18 months field experiment on spiders. However, we contend that
15 critical flaws in their predictions, assumptions, methods and interpretations undermine this claim.
16 The data presented are unreliable and are equally consistent with GS and individual-level
17 selection [ILS]; thus, the authors cannot credibly conclude that GS has produced the observed
18 patterns.

19 **1. Predictions: No critical test of GS**

20 Evaluating GS involves, at a bare minimum, estimating and comparing both individual and
21 group fitness, as stated by previous reviews^{2,3} and performed by other studies^{4,5}. Yet PG14 did
22 not estimate individual fitness, and so cannot evaluate the relative importance of GS compared to
23 ILS. The chosen species, *Anelosimus studiosus*, is solitary, rarely forms groups⁶, and shows no
24 evidence of reproductive restraint or skew within groups⁷. Thus, individual and group fitness are
25 not expected to conflict and are generally confounded, emphasising how crucial it is, firstly, to
26 formulate predictions capable of distinguishing ILS and GS explanations and, secondly, to
27 estimate individual fitness.

28 Both of PG14's predictions could follow equally well from ILS as from GS: Prediction 1)
29 "Compositions [i.e. within-group phenotypic frequencies] that approximate the normal mixtures
30 that characterize each site will enjoy greater success". Merely demonstrating differential survival
31 of groups does not allow the authors to distinguish successful groups from groups of successful
32 individuals. PG14's "group trait" is a group size dependent behavioural polymorphism.
33 Experimental changes in this "group trait" (i.e. manipulating group size and phenotype
34 frequency) may directly affect within-group individual fitness just as well as whole-group
35 fitness⁸⁻¹¹. Specifically, creating experimental groups that deviate from locally stable
36 polymorphisms may reduce mean *individual* fitness, rendering group extinction more likely. The
37 prediction of differential group extinction can therefore result from ILS just as plausibly as from
38 GS. Similarly ambiguous is Prediction 2) "Colonies should only be able to adaptively hone
39 compositions when composed of native individuals". If 'Native colonies' can "adaptively"
40 change phenotype frequencies over time, this may occur via several mechanisms, as PG14
41 mention (plasticity, phenotype-biased dispersal, etc.). Yet, any of these mechanisms may evolve
42 by ILS, a possibility ignored by PG14.

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44 **2. Assumptions: Unreliable selection pressure**

45 PG14's conclusions rest upon the assumption that 'naturally-occurring mixtures' (i.e. field
46 phenotypic frequencies, PG14's Fig. 1a) represent consistent selection pressures across years.
47 Yet, the years of measurement were patchy (2007-2014), differed among sites and often did not
48 overlap (Table 1). Indeed, PG14 sampled significantly different phenotypic mixtures and group
49 sizes among years at each site (mixtures: $p = 1.1 \times 10^{-5}$; group size: $p = 7.8 \times 10^{-14}$; Fisher's
50 combined p -value across separate Kruskal-Wallis tests for each site) but ignored this variation
51 and pooled dissimilar data. In four out of nine samples taken at high resource sites, the selection
52 pressure was no different from zero (non-significant correlations between mixtures and group
53 size; separate lm for each year at each site), and in two out of the three low resource sites the
54 relationship between mixture and group size differed significantly from year to year (Norris
55 Dam: $p = 0.0074$; Don Carter: $p = 0.017$; lm: interaction between log(group size) and year on
56 phenotypic mixtures). Moreover, half of the sites had not been assessed for four to six years.
57 These measurements cannot be assumed to represent consistent, current selection pressures.

58

59 **3. Methods: Unreliable group trait**

60 PG14 infer GS by comparing parental [P] and grand-offspring [F2] generations based on parental
61 traits alone: they compare P-phenotypic compositions of P-colonies with P- (not F2)
62 compositions of surviving F2-colonies (PG14's Fig. 1c is identical to Fig. 1b, minus extinct
63 colonies: F2-colonies are depicted with their grandparents' compositions). It stretches credibility
64 to assume that past compositions are visible to selection but present compositions are not.
65 Indeed, 'Foreign colonies' changed to display F2-compositions in a pattern *opposite* to the
66 assumed selection pressure (Fig. 1; PG14's Fig. 2). These changes mean that F1-compositions
67 presumably also differed from P-compositions (and were visible to selection during that
68 generation) but F1 was not assessed (Fig. 1).

69 Changes *within* generations were also not considered (Fig. 1). Around egg hatching¹², colonies
70 peak in size, after which mortality and dispersal decrease colony size. Phenotypic composition
71 cannot be considered a stable "group trait" when its proposed selective advantage is a function of
72 group size, and group size changes nonlinearly over time. Compounding this, compositions of P
73 and F2 were apparently measured at different developmental stages (Fig. 1): a serious flaw, as
74 individual phenotypes are affected by reproductive status¹³. PG14 compared sexually mature
75 females (P) with grandchildren (F2) that presumably were juvenile, mixed-gender, and receiving
76 maternal care (Fig. 1; mothers die off in October¹²; juvenile spiders are unsexable).

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78 **4. Interpretations: No evidence of evolution**

79 None of their findings supports PG14's puzzling claim to have demonstrated a "marked
80 evolutionary response to GS". Rather, after two generations, surviving 'Foreign colonies' *failed*
81 to change phenotypic compositions in site-appropriate ways (instead changing to express
82 compositions appropriate for their original site), suggesting a lack of genetic change over the
83 experiment (Fig. 1). PG14 argue that this constitutes evidence that mechanisms for adjusting
84 compositions are locally adapted due to historical GS, but provide no justification for this claim:
85 while they provide data suggesting phenotypes themselves may be partially heritable, there is no
86 evidence that this "adjustment mechanism" has undergone genetic change and, again, no attempt

87 to reject ILS as an explanation. 'Foreign colonies' may revert to their native phenotype
88 compositions without genetic change, for example due to persistent maternal or epigenetic
89 effects, either of which may respond to ILS or GS. The evolutionary mechanisms shaping
90 population-level differences, whether in phenotype frequencies or the means by which these
91 change over time, simply have not been addressed in this paper.

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93 We stress that we would welcome any field study demonstrating that GS causes genetic change
94 over generations in ways inconsistent with ILS. Given recent high-profile exchanges over the
95 relative importance of GS^{14,15}, such a paper would be a significant contribution to the field.
96 Unfortunately, PG14 is not such a study.

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98 **Figures and tables**

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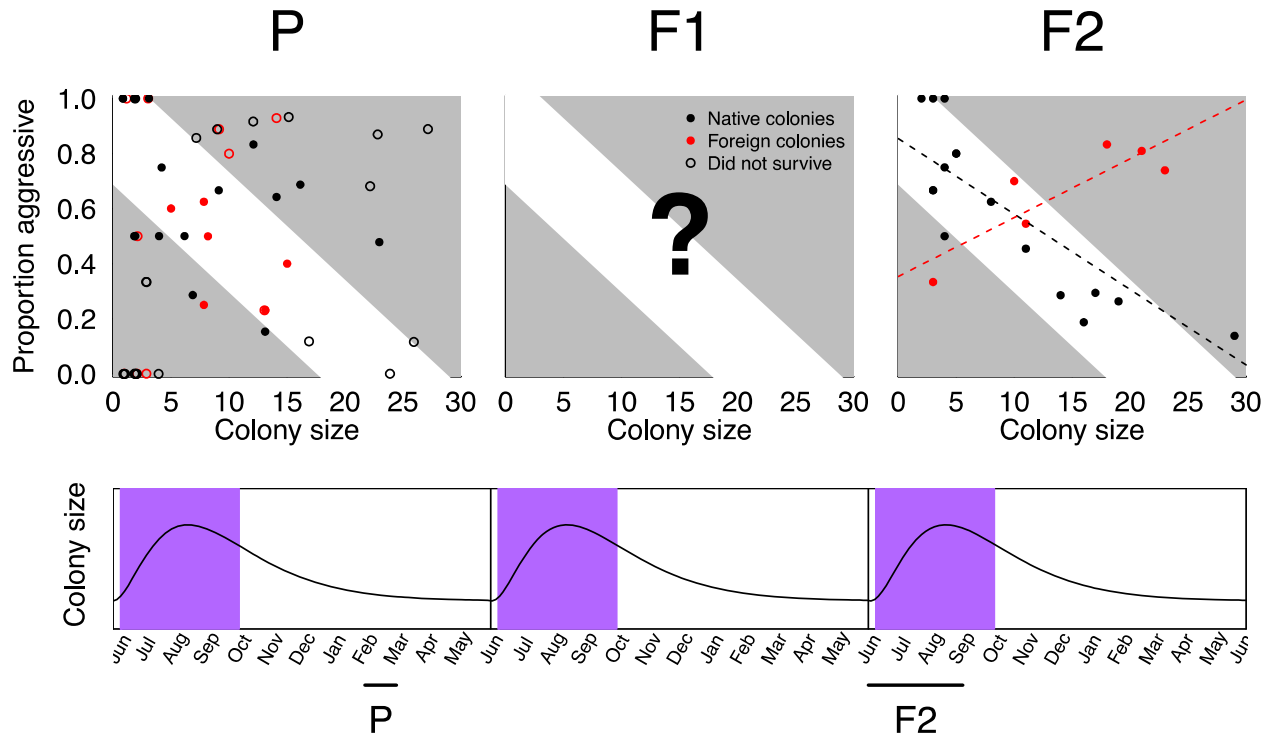
100 **Table 1.** Collection years for each field site. Data from years marked with ‘X’ were pooled
 101 within sites to create the naturally-occurring mixtures (Fig. 1a in PG14) used to infer site-
 102 specific selection pressures. Grey shaded years indicate the years during which the experiment
 103 took place.

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		Collection Years							
Site		2007	2008	2009	2010	2011	2012	2013	2014
High Resource Sites	Melton Hill	X	X	X		X	X		
	Little River	X	X						
	Moccasin Creek							X	X
Low Resource Sites	Norris Dam	X	X	X		X	X		
	Clinch River		X		X				
	Don Carter			X	X				

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108 **Figure 1: Overview of methods and results from PG14**

109 **Upper panel:** Distribution of experimental colonies placed in all of six field sites in the parental
 110 [P] generation; the missing information of the next generation [F1]; and the distribution of final
 111 compositions of the grand-offspring generation [F2]. Phenotypic compositions, i.e. proportions
 112 of aggressive individuals in each colony, are plotted against colony sizes. We present data only
 113 from one low-resource field site, ‘Don Carter’, to illustrate the setup. Black dots represent
 114 ‘Native colonies’ (created with spiders collected at Don Carter); red dots represent ‘Foreign
 115 colonies’ (spiders collected at high-resource field site ‘Moccasin Creek’). Full circles of both
 116 colours in P are colonies that were still alive in F2 (equal to PG14’s Fig. 1c); empty circles are
 117 colonies that had gone extinct by F2. The white band represents the proposed selection pressure
 118 at that field site: a regression line fitted on phenotypic compositions and colony sizes of
 119 ‘naturally-occurring’ colonies at Don Carter (based here on colonies of sizes up to 30; its
 120 thickness chosen arbitrarily). Dotted lines in F2 represent regressions of the final F2-
 121 compositions of the surviving colonies: ‘Native’ (black) versus ‘Foreign’ (red). Surviving
 122

123 colonies had P-compositions close to the white 'selection band', but F2-compositions differed
124 according to site of origin: 'Native' F2-compositions were close to the selection band while
125 'Foreign' F2-compositions followed a positive regression, dissimilar to the selection band. Setup
126 and results were similar in the two additional low-resource sites while the three high-resource
127 sites showed opposite trends (i.e. selection bands were positive regression lines while the
128 'Foreign' F2-regressions showed negative correlations). *Note that although 'Foreign colonies'*
129 *end up opposite to the proposed selection pressure, PG14 still conclude that their proposed*
130 *selection pressure was supported. Lower panel* shows how the size of an *A. studiosus* colony is
131 expected to vary within years with a peak around egg hatching during summer. The period of
132 maternal care is marked in purple. Black lines marked with 'P' and 'F2' indicate at which point
133 in the life cycle PG14 performed the behavioral assays to determine the phenotypic compositions
134 of colonies. *Note that group sizes and phenotypic compositions of P and F2 were measured at*
135 *different points, comparing sexually mature females (P) with juvenile grandchildren (F2) during*
136 *maternal care at a stage where offspring sex cannot be determined.*

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P

F1

F2

