1   # MULTILEVEL SELECTION IN GROUPS OF GROUPS

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Abstract

11

12   Natural selection occurs at many levels. We evaluated selection acting on collectives at a level of

13   multilevel selection analysis not yet quantified: within and between clusters of groups. We did so

14   by monitoring the performance of natural colonies of social spiders with contrasting foraging

15   aggressiveness in clusters of various sizes. Within-clusters, growth rates were suppressed when

16   colonies were surrounded by more rival groups, conveying that competition is greater. When

17   colonies were surrounded by few rivals, the more aggressive colonies in a cluster were more

18   successful. In contrast, relatively non-aggressive colonies performed better when surrounded by

19   many rivals. Patterns of selection between-clusters depended on the performance metric

20   considered, but cluster-wide aggressiveness was always favored in small clusters. Together,

21   selection both within- and between natural clusters of colonies was detectable, but highly

22   contingent on the number of competing colonies.

23   **Keywords**: collective behavior, group selection, indirect genetic effects, multilevel selection,

24   social selection

25

26   Natural selection is context-dependent. In social organisms, context-dependent selection in

27   individual-level traits forms the basis of diverse and powerful evolutionary forces, like indirect

28   genetic effects, social selection, frequency-dependent selection, and their intersections (*1-5*). We

29   contend that the context-dependent nature of selection observed in individual traits could

30   transcend to the level of the group and their collective behavior too. For instance, the collective

31   traits that enable group success might depend on the presence and phenotypes of surrounding

32   groups, resulting in cases where the phenotypic compositions of some clusters of groups

33   outperform other clusters. Whether selection occurs above the level of the group is not often

34   evaluated in the multilevel selection literature (*6-9*), and the idea is often viewed critically on

35   conceptual grounds. Field data documenting such selection in intact free-living systems are

36   particularly rare. However, if selection above the level of the group is present, then it has the

37   potential to change the evolution of individual and collective traits across a variety of social

38   organisms and communities, especially in systems where social groups interact with each other

39   intensely.

40

41   Here we evaluate how the number of neighboring rival groups changes two tiers of selection on

42   collective behavior in a social spider (*Stegodyphus dumicola*). Specifically, we predict that the

43   intensity of competition experienced by a focal group will scale positively with the number of

44   rival groups nearby, which could alter selection both within and among clusters of colonies and

45   potentially select against clustering (*10*). Prior work on *S. dumicola* showed that frequency-

46   dependent selection can, at least in principle, act on among-group differences in collective

47   behavior (*11*). In experimental clusters of colonies, the success of aggressive groups decreases as

48   they become common within clusters. This is because aggressive colonies are more sensitive to

49 low resource conditions, and prey are scarcer in clusters dominated by aggressive colonies. We

50 therefore predicted that i) increasing competition between colonies (i.e., increasing the number

51 of rivals) will reduce the performance of aggressive colonies, and ii) the performance of

52 aggressive clusters of colonies will decrease as the number of colonies in the cluster increases.

53

54 To examine the degree of selection on collective behavior within and among clusters of spider

55 colonies, we monitored the foraging phenotypes and performance of colonies from March 2018

56 to March 2019. Natural clusters of colonies (1-9 colonies, Fig. 1a) were identified along road

57 side fences February-March 2018 in South Africa. We evaluated the collective foraging

58 aggressiveness and colony size of each colony within each cluster. Foraging aggressiveness was

59 evaluated thrice over two days in 2018 and 2019 by counting the number of individuals that

60 responded to simulated prey item in the web. Starting colony sizes were estimated using the

61 volume of the colony's nest (i.e., a disk-shaped cylinder), which is tightly associated with the

62 number of spiders in the colony for fence-dwelling colonies (r^2= 0.72-0.85, varying slightly

63 among years). Variation in collective foraging aggressiveness is repeatable within and across

64 generations in *S. dumicola*, and colony differences are transmitted from parent to daughter

65 colonies during fission events, which create local clusters of related colonies upon which

66 selection could act (*12*). This parent-offspring resemblance is itself retained across years (*11*).

67 Thus, this system fulfills the rare pre-conditions necessary for a phenotypic response to selection

68 both within and among clusters of colonies.

69

70 Colony success was determined at two time points. In June 2018, when colonies are dormant

71 during the Austral winter, nests were removed from their substrate and dissected at hotels to

4

72   count the number of egg cases produced by the colony in 2018 (*egg case #*). Colonies were then

73   re-adhered to their former positions using staples and aluminum wiring to supplement supporting

74   structure. In March 2019, we destructively recollected colonies, dissected them open again, and

75   counted the number of females within each nest. This allowed us to record the change in colony

76   size from one generation to the next (Δ *colony size*).

77

78   Colonies in larger clusters were less successful, and this was true for both change in colony size

79   (*main effect* = -0.171 $\pm$ 0.05; p = 0.001) and per capita egg cases (*main effect* = -0.116 $\pm$ 0.06; p

80   = 0.05). This finding confirms prior correlative and experimental findings that colonies of social

81   spider compete (*11, 13, 14*), and conveys that larger clusters exacerbate competition for prey

82   resources. Thus, selection should disfavor high levels of aggregation and promote greater

83   dispersal, especially when clusters are composed of related colonies, which is not uncommon in

84   *S. dumicola (12)*.

85

86   Selection on within-cluster collective aggression was dependent on cluster size. In small clusters

87   of colonies, higher aggressiveness relative to neighboring colonies was favored, whereas non-

88   aggressive colonies were favored in large clusters. This was true both for colonies' proportional

89   change in size (Fig. 1b; *interaction*: -0.336 $\pm$ 0.06, LRT = 30.05, p < 0.001, *main effect*: 0.171 $\pm$

90   0.057), and *per capita* egg case production (Fig. S1; *interaction*: -0.175 $\pm$ 0.036, LRT = 25.33, p

91   < 0.001, *main effect*: 0.314 $\pm$ 0.035). Selection on aggressiveness is thus density-dependent,

92   switching from positive in small clusters to negative in larger clusters, likely as a result of

93   increased resource competition in large clusters because aggressive colonies are known to

94   outcompete docile colonies if resources are plentiful (*11*). Density-dependent social or multilevel

95   selection has been observed in both plant and animal systems (*15-18*). However, the findings

96   here are somewhat unique, first because the direction of selection is completely reversed in a

97   density-dependent manner and not simply magnified, and second, because the density-

98   dependence occurs at the level of the colony and their collective behavior rather than individual

99   level traits.

100

101   We detected selection among clusters of colonies as well. Clusters of aggressive colonies grew

102   proportionally more if the cluster was small, while this pattern was reversed in large clusters (Fig

103   1c; *interaction*: -0.161 ± 0.074, LRT = 4.655, p = 0.031, *main effect*: 0.006 ± 0.075). For *per*

104   *capita* egg cases production, however, more aggressive clusters were always more successful,

105   regardless of cluster size (Fig. 1d; *interaction*: -0.045 ± 0.095, LRT = 0.261, p = 0.61, main

106   effect = 0.149, se = 0.056, LRT = 25.334, p < 0.001). Thus, the advantage accumulated by large

107   and aggressive clusters until egg case production is possibly offset by costs following the

108   emergence of the next generation. Alternatively, the selective advantage of large aggressive

109   clusters could continue across generations but be hidden by increased long-distance dispersal

110   from these clusters, potentially linked to local prey resources (*19*). The long-distance dispersal

111   abilities of this species prevent us from discriminating between these interpretations. However,

112   the possibility for conflicting selection within vs. among clusters at large cluster sizes cannot be

113   ignored for now: non-aggressive colonies produce more egg cases than their many neighbors, but

114   large neighborhoods of aggressive colonies are still more fecund in aggregate (Fig. S1). What

115   becomes of this cluster-level advantage is unclear.

116

117   Despite more than a half-century of scientific debate on the efficacy of selection above the level

118   of the individual and dozens of papers detecting its presence, multilevel selection remains one of

119   the most instantly controversial topics in evolutionary biology. Here were use multilevel

120   selection analysis to evaluate selection within and across two levels of selection, both of which

121   occurs above the level of the individual: i) groups and ii) groups (clusters) of groups. The *S.*

122   *dumicola* system is suited for such an analysis because between-group differences in collective

123   behavior are transmitted with fidelity down colony lines, and because the differences in colony

124   aggressiveness cannot be linearly traced back down to the phenotypes of individual constituents.

125   Collective behavior is instead determined by a highly non-additive process that depends on

126   keystone individuals (*20*), social network structure (*21*), and a colony's social history (*22*). Yet,

127   our data provide evidence that selection at the level of colonies and beyond are readily detectable

128   in free-living colonies of this species, and that these levels of selection are potentially at odds for

129   some cluster sizes. It therefore appears that evolutionary processes that emerge from feedback

130   (positive or negative) between traits and the competitive environment are not restricted to

131   individual levels traits, theoretical considerations, or contrived experimental settings. They are

132   perhaps instead reasonably common and generalizable features of selection that scale from the

133   level of genes to neighborhoods of competing societies, and conceivably beyond.

134

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203

204 *Natural History*

205 Social *Stegodyphus* live in multifemale colonies that cooperate in alloparental care, web

206 maintenance, and cooperative hunting (*23, 24*). Colonies are usually founded what one or a few

207 pre-mated females disperse to found a new colony, which grows by the breeding of brothers to

208 sisters for multiple generations. Only a small proportion of males move between colonies, which

209 results in high levels of relatedness within groups, and variable levels of relatedness between

210 neighboring groups (*12, 23, 24*).

211

212 *Cluster Identification*

213 We searched for colonies of *S. dumicola* positioned along fences (highways R74 and R714) by

214 driving long stretches of highway. Clusters were defined as groups as nests that resided within

215 1m of another nest, but where the majority of the capture web was not shared with other nests.

216 We focused on fence-dwelling colonies because 1) colonies on fences outperform tree-dwelling

217 colonies, 2) the largest and densest clusters reside on fences, and 3) fence-dwelling nests are

218 two-dimensional (plate-shaped), which eases their dissection and reassembly (*25*). In March

219 2019, we destructively recollected colonies, dissected them open again, and counted the number

220 of females within each nest (*colony size*). Some focal clusters vanished entirely during the study

221 from unknown causes. Only clusters that were tracked both years are considered here.

222

223 *Nest Volume & Group Size*

224 We used nest volume to estimate the starting colony sizes of the colonies in the present study.

225 Nest volume was estimated using a cylinder formula, with the colony's radius and depth

226 measured in situ using standard tape measurer or digital calipers, if the colony was small enough.

10

227   Using nest volume as a proxy for groups size is not uncommon in the social spider literature, and

228   nest volume provides only a coarse approximation of colony size in social *Stegodyphus* when

229   they do not reside on fences ($r^2 = 0.35$-0.69) (*26*). Therefore, in March 2018 we estimated the

230   volume of 37 fence-dwelling colonies and then counted the number of adult female spiders

231   therein. We found that nest volume explained 83.5% of the variation in colony size in these

232   colonies, and that ending nest volume explained 72% of the variation in our focal colonies here.

233   Thus, nest volume provides a more precise estimate of colony size in fence-dwelling *S.*

234   *dumicola*, presumably because fences are more homogeneous environments for colony creation

235   and expansion.

236

237   *Collective Foraging Aggressiveness*

238   Colonies' foraging aggressiveness was estimated by the number of attackers deployed during a

239   staged encounter with prey (after *11, 27*). Trials were initiated by placing a 1 cm x 1 cm square

240   of computer paper in the capture web, and then vibrating the piece of paper for three minutes or

241   until the spiders made contact with the paper and seized it. The paper was vibrated using a

242   handheld vibratory device with a thin aluminum prod extending from one end. The vibrating

243   prod was then placed gently against the paper, causing is to flitter back and forth. We then

244   counted the number of spiders that emerged in response to this vibratory stimulus. Colony

245   foraging aggressiveness was evaluated thrice in 2018, twice in one day and a third time a day

246   later. In 2019, surviving colonies were assayed 1-3 times more, to evaluate whether between-

247   group differences in collective behavior were associated across generations, which they were

248   ($F_{1,99} = 99.52$, main effect = 0.96, se $\pm$ 0.10, p < 0.0001). The majority of colonies were assayed

249   in an identical manner to 2018. However, for a small subset of colonies, foraging aggressiveness

11

250   was measured only once in 2019 because the field season was cut short owing to a medical

251   emergency.

252

253   *Removal & Dissection*

254   In June 2018, when colonies are dormant during the Austral winter, nests were removed from

255   their substrate and dissected at hotels to count the number of egg cases produced by the colony in

256   2018 (*egg case #*). Colonies were refrigerated during this process as to keep spiders inactive

257   prior to and after the dissection process. The perimeter of the nest was then stapled closed and

258   colonies were re-adhered to their collection points using aluminum wiring to supplement their

259   supporting structures and staples.

260

261   *Statistical Methods*

262   To separate colony aggression into within cluster and among cluster components, we calculated

263   the mean aggression of colonies within each cluster in 2018 (giving "cluster average

264   aggression"), and then subtracted this from each colony's mean aggression score in 2018 (giving

265   "colony Δ aggression"). This approach for separating levels of selection is known as "contextual

266   analysis" (*28, 29*). We then entered each of these terms into a linear mixed-effect model with a

267   Gaussian error distribution, with the proportion increase in size of the colony between 2018 and

268   2019 as the response variable. For this measure of colony success, colonies that went extinct

269   have a score of 0, while colonies that doubled in size have a score of 1, scores ranged from 0 to

270   2.826. The effect of colony Δ aggression corresponds to within-cluster selection, while the effect

271   of cluster average aggression corresponds to among-cluster selection. We added the number of

272   colonies in the cluster as a fixed effect and interacted this term with both cluster average

273   aggression and colony delta aggression, to determine if either level of selection was density

274   dependent. All three of these fixed effects were mean-centered and divided by their own standard

275   deviation, meaning each variable has a mean of 0 and a variance of 1, making the regression

276   coefficients easier to interpret (*30*). Cluster ID was entered as a random effect. We tested each

277   interaction term for significance using likelihood ratio-tests, and if it was not significant at $\alpha =$

278   0.05, we removed it and tested the main effect in the same manner.

279   To confirm our findings were robust to the choice of colony performance metric, we re-

280   fitted this model with the number of egg-cases in the colony as a response variable, an offset of

281   the log of colony size in 2018 (effectively modelling egg cases per capita) and a Poisson error

282   distribution. Models were fitted in R 3.5.3 (*31*) using the package "glmmTMB" (*32*).

283

13
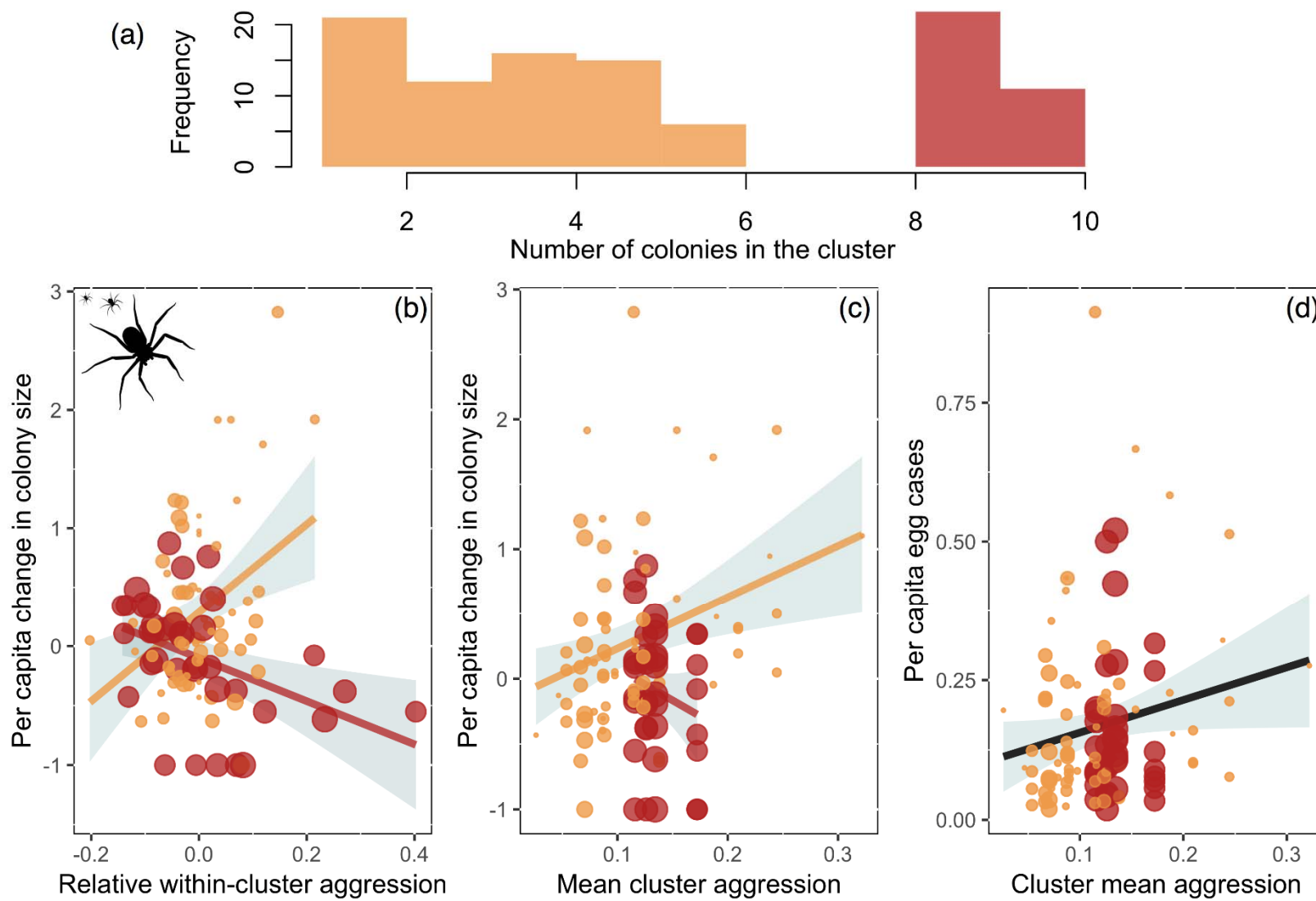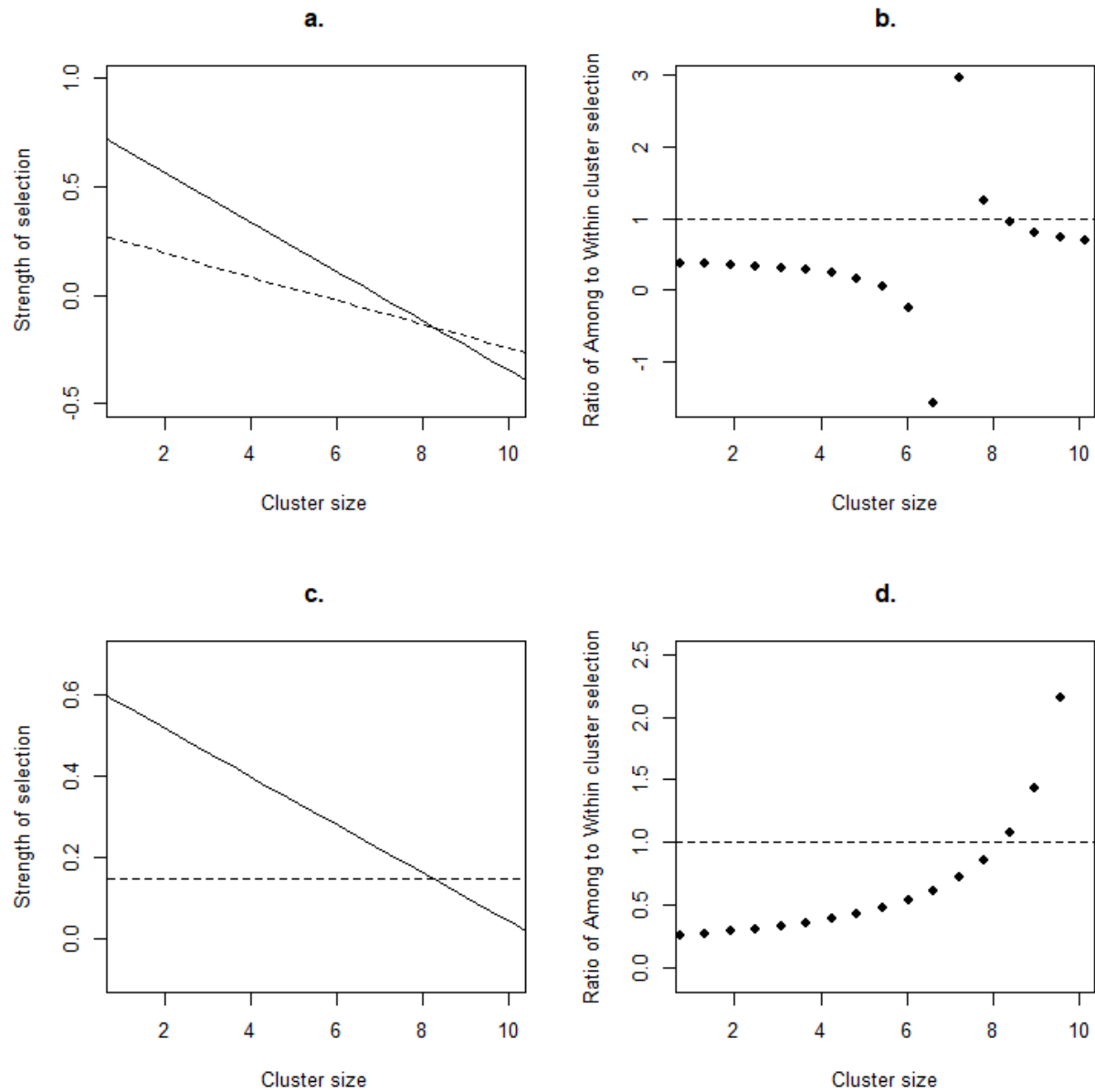
Figure 1

Figure S1: Top panels (a & b) depict selection estimates based on change in colony size, and the bottom panels (c & d) depict selection based on egg case production. The left panel (a & c) depicts the strength of within (solid line) and among (dashed line) cluster selection at various cluster sizes. The right panel (b & d) is how the ratio (among/within) changes with cluster size.