1   **Mother knows best: maternal egg-clutch size predicts larval group size in pine sawflies (genus**

2   ***Neodiprion*)**

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8   Keywords: parent-offspring conflict, social behavior, phylogenetic comparative methods,

9   coevolutionary arms race, antagonistic coevolution

10   Submission Type: Article

11   Word Count: 4417

12   Other Elements: "Mother Knows Best Supplemental Information"

13

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19   **Abstract**

20   Evolutionary conflicts are pervasive in nature and have the potential to drive antagonistic coevolution of

21   conflict-related traits. However, when such conflicts are weak or idiosyncratic, phenotypic signatures of

22   coevolutionary arms races may be absent. Here, we ask whether variation in group-living traits among

23   pine-sawfly species in the genus *Neodiprion* is consistent with a history of parent-offspring conflict. To

24   address this question, we compile data on adult female clutch size, larval aggregation behavior, and

25   larval group size for a monophyletic group of 19 eastern North American *Neodiprion* species from field

26   observations, laboratory assays, and published descriptions. We then evaluate the extent to which each

27   trait exhibits phylogenetic signal and, based on these results, examine correlations between group-size

28   traits both with and without phylogenetic correction. Although female oviposition behavior and larval

29   grouping behavior varies among species and variation in these traits is decoupled from phylogeny, we

30   find no evidence of antagonistic coevolution between these traits. Furthermore, while larvae are

31   physically capable of dispersal, female clutch size is a strong predictor of larval colony size, indicating

32   that larvae do not substantially alter initial group size after hatching. Thus, although theoretical work

33   demonstrates the potential for parent-offspring conflict over group size in animals that lack parental

34   care, our data suggest that this type of conflict is not likely to be a long-term driver of phenotypic

35   evolution.

Introduction

36

37   Evolutionary theory predicts that conflict—scenarios in which interacting entities have divergent

38   fitness optima for some shared trait of interest—is widespread in nature (Queller and Strassmann 2008).

39   Conflict manifests in a variety of contexts, including between males and females (sexual conflict;

40   (Chapman et al. 2003; Arnqvist and Rowe 2005), parents and their offspring (parent-offspring conflict;

41   (Trivers 1974; Godfray 1995; Crespi and Semeniuk 2004)), and within the genome of a single individual

42   (intragenomic conflict; (Burt and Trivers 2009; Garder and Úbeda 2017)). Regardless of the context,

43   evolutionary conflicts have the potential to fuel antagonistic coevolution, in which there is reciprocal

44   evolutionary change in the genes and traits that mediate the conflict (Rice 1996; O'Neill et al. 2007;

45   Carmona et al. 2015; Lindholm et al. 2016; Dougherty et al. 2017). When this coevolutionary arms race

46   plays out over the course of lineage diversification, it can give rise to correlated evolution of conflict-

47   related traits (Briskie et al. 1994; Arnqvist and Rowe 2002; Lloyd and Martin 2003; Koene and

48   Schulenburg 2005; Kölliker et al. 2005; Ronn et al. 2007) and elevated rates of diversification (Arnqvist et

49   al. 2000; Zen and Zen 2000; Kraaijeveld et al. 2011; Crespi and Nosil 2013). Thus, a comparative

50   approach is a potentially powerful tool for making inferences about the strength and long-term

51   evolutionary consequences of conflict.

52   One type of conflict that has received theoretical attention, but minimal comparative study, is

53   conflict between parents and offspring over offspring group size. Whenever siblings compete for limited

54   resources, a female's optimal clutch size can differ from that of her offspring (Godfray 1987; Parker and

55   Mock 1987; Godfray and Ives 1988; Damman 1991; Godfray et al. 1991; Desouhand et al. 2000). This

56   type of parent-offspring conflict could occur even in the absence of any parental care (Costa 2018). For

57   example, siblicide among parasitoid larvae may constrain females to produce clutches smaller than

58   would be otherwise optimal (Godfray 1987; Parker and Mock 1987; Rosenheim 1993). Similarly,

59   competition among herbivorous, gregarious insect larvae may favor a reduction in egg-clutch size of

60   ovipositing females (Godfray and Parker 1992). Conversely, when larvae disperse readily—as is the case

61   in many grazing insects—egg clustering or dumping may be favored (Roitberg and Mangel 1993). Under

62   this scenario, there can be parent-offspring conflict over larval dispersal tendencies (Roitberg and

63   Mangel 1993; Sjerps and Haccou 1994). Thus, across a wide range of invertebrate life histories, parent-

64   offspring conflict has the potential to drive coevolution between female oviposition behavior (clutch

65   size) and offspring behaviors that mediate group dynamics (e.g., siblicide, non-lethal aggression, and

66   dispersal tendency).

67   Following Arnqvist and Rowe (2002), who described coevolutionary dynamics between traits

68   that mediate sexual conflict, we can make three predictions regarding the evolution of traits that

69   mediate group-size conflict. First, because antagonistic coevolution can produce rapid evolutionary

70   change, parent-offspring conflict over group size should generate extensive interspecific variation in

71   group-living traits. Second, because coevolutionary arms races are expected to have bouts of escalation

72   and de-escalation (Parker 1983; Härdling 1999), trait evolution should be decoupled from phylogenetic

73   history (minimal "phylogenetic inertia", (Losos 1999; Arnqvist and Rowe 2002)). Third, assuming that the

74   female optimum clutch size consistently exceeds the offspring optimum clutch size (Godfray 1987;

75   Parker and Mock 1987; Godfray and Parker 1992; Roitberg and Mangel 1993; Godfray 1995), increases

76   in female egg-clutch size should favor offspring behaviors that either increase competitive ability or

77   decrease colony size (e.g., via dispersal) and vice versa, generating a negative correlation between these

78   traits among species. Although analogous predictions have been confirmed in lineages experiencing

79   sexual conflict (Arnqvist and Rowe 2002), they remain untested in the context of group-size conflict (but

80   see: Mayhew 1998).

81   To test these predictions, we take advantage of a group of herbivorous insects for which

82   extensive ecological data are available. The sawfly genus *Neodiprion* (order: Hymenoptera; family:

83   Diprionidae) is a Holarctic group of approximately 50 species specialized on host plants in the family

84   Pinaceae. Within this genus, the *"Lecontei* clade" is a monophyletic lineage of 21 eastern North

85   American species well studied from taxonomic (Ross 1955; Ross 1961; Linnen and Smith 2012),

86   phylogenetic (Linnen and Farrell 2007; Linnen and Farrell 2008a; Linnen and Farrell 2008b; Linnen and

87   Farrell 2010), and life history and behavioral perspectives (Coppel and Benjamin 1965; Knerer and

88   Atwood 1973; Knerer 1993; Costa and Louque 2001; Fowers and Costa 2003; Terbot II et al. 2017).

89   Importantly, eastern North American *Neodiprion* have documented variation in female egg-laying

90   behavior (Supplemental Table 1) and larval grouping behavior (Terbot II et al. 2017, Supplemental Table

91   1). A more detailed summary of relevant life history and ecology of *Neodiprion* sawflies can be found in

92   the supplemental materials.

93   If parent-offspring conflict over group size has shaped the evolution of clutch-size traits, trait

94   variation should match the three predictions outlined above: extensive interspecific variation, minimal

95   phylogenetic signal, and a negative correlation between female egg-clutch size and larval grouping

96   behavior among species. To evaluate these predictions, we combined our own observations from the

97   field and the lab with published descriptions of female and larval behavior from 19 of the 21 eastern

98   North American *Neodiprion* species. To gain additional insight into our data, we also examined

99   correlations between female and larval behavior and larval colony size. If larval behaviors tend to modify

100   initial egg-clutch size, we would expect positive correlations between larval grouping behaviors and

101   larval colony size. Conversely, if there is minimal post-hatching modification of the initial clutch size, we

102   would expect a stronger correlation between female clutch size and larval colony size.

103

104   **Materials and Methods**

105   **Characterizing variation in group-size traits**

106   We compiled data on female egg-clutch size, larval grouping behavior (aggregative tendency),

107   and larval colony size for 19 of 21 eastern North American *Neodiprion* species (Linnen and Farrell 2008a,

108   b). The only two species for which we were unable to obtain data were *N. cubensis* and *N. insularis*, both

109   endemic to Cuba. Here, we briefly describe how we compiled data for each trait. Additional details are

110   provided in the Supplemental Tables 1-4.

111   To estimate egg-clutch size for each species, we combined field observations, laboratory assays,

112   and published descriptions. Field observations took place in 2002, 2015, and 2016 and consisted of

113   counts of egg scars on needles found in close proximity to early instar larvae that were collected and

114   reared to later instars or adults for identification. Clutch sizes were estimated from the number of egg

115   scars found on individual branch termini. To supplement our field observations, we used laboratory

116   assays to obtain clutch-size estimates for several species. In each assay, we released a lab-reared, virgin

117   female into a mesh cage (35.6 x 35.6cm x 61cm) containing four *Pinus* seedlings. To obtain an average

118   clutch size for each female, we recorded the number of eggs laid in each discrete clutch (i.e., a single

119   branch terminus on a single seedling). For these assays, females were reared via methods described

120   elsewhere (Harper et al. 2016) and provided seedlings from the *Pinus* species on which they (or their

121   wild-caught progenitors) were originally collected. Finally, to supplement our own clutch-size data, we

122   surveyed the literature and included published data from which we could infer mean clutch size. In total,

123   we obtained clutch-size data for 16 of the 19 *Neodiprion* species included in our study (Supplemental

124   Tables 1-2).

125   To measure larval aggregative tendency, we used a behavioral assay described in Terbot II et al.

126   2017. Briefly, we spaced five larvae of a particular species equidistantly along the perimeter of a 14.5 cm

127   diameter petri dish and recorded their behavior for 90 minutes using a Lenovo Ideapad laptop and

128   either a Logitech or Microsoft webcam. We then used the program Video Image Master Pro (A4Video

129   2016) to extract individual video frames and a custom Java application to extract one image still per

130   every 3 minutes of video (Terbot II et al. 2017). For each extracted image, we measured all pairwise

131   distances between each of the five larval heads. To provide an acclimation period for the larvae to

132   adjust to the petri dish arena, we excluded the first 12 images (36 minutes) of each video (Terbot II et al.

133   2017) using Microsoft Excel 2013 (Microsoft 2012) and a custom Perl script. This processing pipeline

134   produced a single mean pairwise larval distance measure for each video. Larger mean pairwise distances

135   correspond to a greater average distance between larvae and therefore a lower "larval aggregative

136   tendency" (i.e., a greater tendency to leave the group); smaller values correspond to a smaller average

137   distance and a higher "larval aggregative tendency". All larvae used in these assays were collected in the

138   field, then reared in the lab on their natal host species via standard rearing protocols until they reached

139   a suitable size for videotaping. To reduce developmental noise in aggregative behavior, only late-instar,

140   feeding larvae were assayed (Terbot II et al. 2017). Collection information and other details regarding

141   the aggregative tendency assays are included in Supplemental Tables 3-4. In total, we measured larval

142   aggregative tendency in 19 eastern North American *Neodiprion* species.

143   To obtain estimates of larval colony size for each species, we combined our own field

144   observations with published descriptions of *Neodiprion* colony size. Field observations, which were

145   recorded between 2002-2004 and 2013-2016, consisted of approximate counts of the number of larvae

146   found in each collected colony. Colonies were located in the field via a combination of visual searching

147   and the use of beating sheets (i.e., tapping on branches and catching dislodged larvae on a large, square

148   piece of fabric). With this combination of methods, we were able to collect both large, conspicuous

149   colonies and isolated individuals. We considered sawflies to be in the same colony if they formed a

150   distinct cluster of larvae (e.g., on the same branch tip) that was spatially isolated from other such

151   groups. We also noted when larvae were collected as isolated individuals (i.e., colony size = 1). To

152   supplement these field data, we included observations from published papers. In total, we obtained

153   group-size data for 19 *Neodiprion* species.

154   To ensure that our data were normally distributed prior to statistical analysis, we applied a van

155   der Waerden rank-based inverse normal transformation to all three datasets. We also multiplied each

156   value in the aggregative tendency dataset by -1 to ensure that for all three datasets, higher values

157   always corresponded to "large group size" phenotypes (larger egg-clutches, higher aggregative

158   tendency, larger larval colony sizes). We used Microsoft Excel 2013 for all transformations. To determine

159   whether species differed in trait values after accounting for variation within species, we used JMP 12

160   (SAS Institute 2016) to perform an analysis of variance (ANOVA) on each of the three datasets. Finally,

161   for use in regression analyses, we calculated mean values for each species and trait. The final data set

162   used in the analysis can be found in [REMOVED FOR DOUBLE BLIND REVIEW].

Evaluating phylogenetic signal for group-size traits

164   To evaluate the extent to which each group-size trait exhibits phylogenetic signal, we estimated

165   Pagel's λ for each trait using BayesTraits v 3.0 (Meade and Pagel 2017). Pagel's λ is a scaling parameter

166   that describes how well phylogenetic relationships predict trait covariance among species, with λ=1

167   corresponding to a model in which a trait evolves via Brownian motion along a phylogeny (i.e., strong

168   phylogenetic signal) and λ=0 corresponding to a star-phylogeny model in which a trait evolves

169   independently for each species (Pagel 1999). For each trait, we used BayesTraits to estimate the

170   likelihood of our data under the "Continuous: Random Walk (Model A)" and three different λ values

171   (λ=0, λ=1, estimated λ). Then, to evaluate the significance of the observed phylogenetic signal, we

172   performed likelihood ratio tests (LRTs; LR = -2ΔlnL; LR is χ^2-distributed with 1 d.f.) in which we compared

173   the likelihood of models in which Pagel's λ was fixed (λ=1 or λ=0) to more complex models in which λ

174   was estimated from the data. To account for phylogenetic uncertainty, we performed LRTs for each tree

175   in sample of 15,000 trees from the posterior distribution of a Bayesian analysis described in Linnen and

176   Farrell 2008a. Prior to analysis, branch lengths on each tree were scaled to time using r8s version 1.71

177   [58], as described in Linnen and Farrell 2010. To account for variation in sample size among traits (N = 16

178   for egg-clutch size, N = 19 for aggregative tendency and larval colony size), we repeated this analysis on

179   reduced (N = 16) aggregative-tendency and larval-group-size datasets.

180   **Evaluating relationships between group-size traits**

181   Because evidence for phylogenetic signal was mixed (see results), we evaluated correlations

182   between group-size traits both with and without phylogenetic correction. For uncorrected analyses, we

183   performed correlation analyses and linear regressions in JMP 12. To test the prediction that conflict

184   generates correlated evolution between parent and offspring traits, we estimated Pearson's correlation

185   coefficient I and evaluated its significance via comparison to Student's *t*-distribution. To determine

186   which traits contribute to larval colony size, we used linear regression to model the relationship

187   between larval colony size (dependent variable) and different combinations of explanatory variables

188   (egg-clutch size, larval aggregative tendency, and their interaction). To compare these models, we

189   calculated the Aikaike information criteria (AIC_c) for all possible models. We then calculated the AIC_c

190   difference between each model and the model with the lowest AIC_c score. We also calculated Aikaike

191   weights and evidence ratios for each model and for the subset of models that had AIC_c differences less

192   than or equal to 5. We used JMP 12 to calculate the AIC_c scores, generate model parameter values,

193   evaluate significance of specific model parameters, and calculate model significance compared to a null

194   model. To calculate AIC_c differences, Aikaike weights, and evidence ratios, we used Microsoft Excel

195   2013.

196 To account for possible phylogenetic constraints on trait evolution, we repeated correlation and

197 regression analyses in BayesTraits v3.0. First, to evaluate the relationship between egg-clutch size and

198 aggregative tendency, we computed the likelihood of the data under the "Continuous: Random Walk

199 (Model A)" model with correlation either assumed or set to zero. To evaluate the significance of the

200 correlation, we used a LRT to compare the models with and without the correlation. Second, to evaluate

201 the contribution of egg-clutch size and aggregative tendency to larval colony size, we used the

202 "Continuous: Regression" model (Organ et al. 2007), with colony size as the dependent variable and egg-

203 clutch size, larval aggregative tendency, and egg-clutch size + larval aggregative tendency as the

204 explanatory variables. To evaluate the significance of the regression models, we performed LRTs that

205 compared models with and without the explanatory variables. For all correlation and regression

206 analyses, we set $\lambda=1$. As described above, we accounted for phylogenetic uncertainty by performing all

207 analyses on a set of 15,000 ultrametric trees sampled from the posterior distribution of a previous

208 MCMC analysis (Linnen and Farrell, 2008a; Linnen and Farrell 2010).

209

210   **Results**

211   **Variation in group-size traits**

212   Egg-clutch size, larval aggregative tendency, and larval colony size varied both between and

213   within species (Fig. 1). Although there was substantial intraspecific variation in some traits/species,

214   ANOVAs revealed significant interspecific variation in all three traits (egg-clutch size: $F_{15, 59}$ = 4.8850, p <

215   0.0001; larval aggregative tendency: $F_{18, 377}$ = 5.3260, p < 0.0001; larval colony size: $F_{18, 360}$ = 17.5712, p <

216   0.0001;)

217   **Figure 1. *Neodiprion* species vary in multiple group-size traits.** Phylogenetic

218   relationships for 19 eastern North American *Neodiprion* are from (Linnen and Farrell

219   2008a; Linnen and Farrell 2010). Box plots depict intraspecific variation for egg-clutch

220   size, larval aggregative tendency, and larval colony size. For all three traits, there is

221   significant interspecific variation (ANOVAs, P<0.001).

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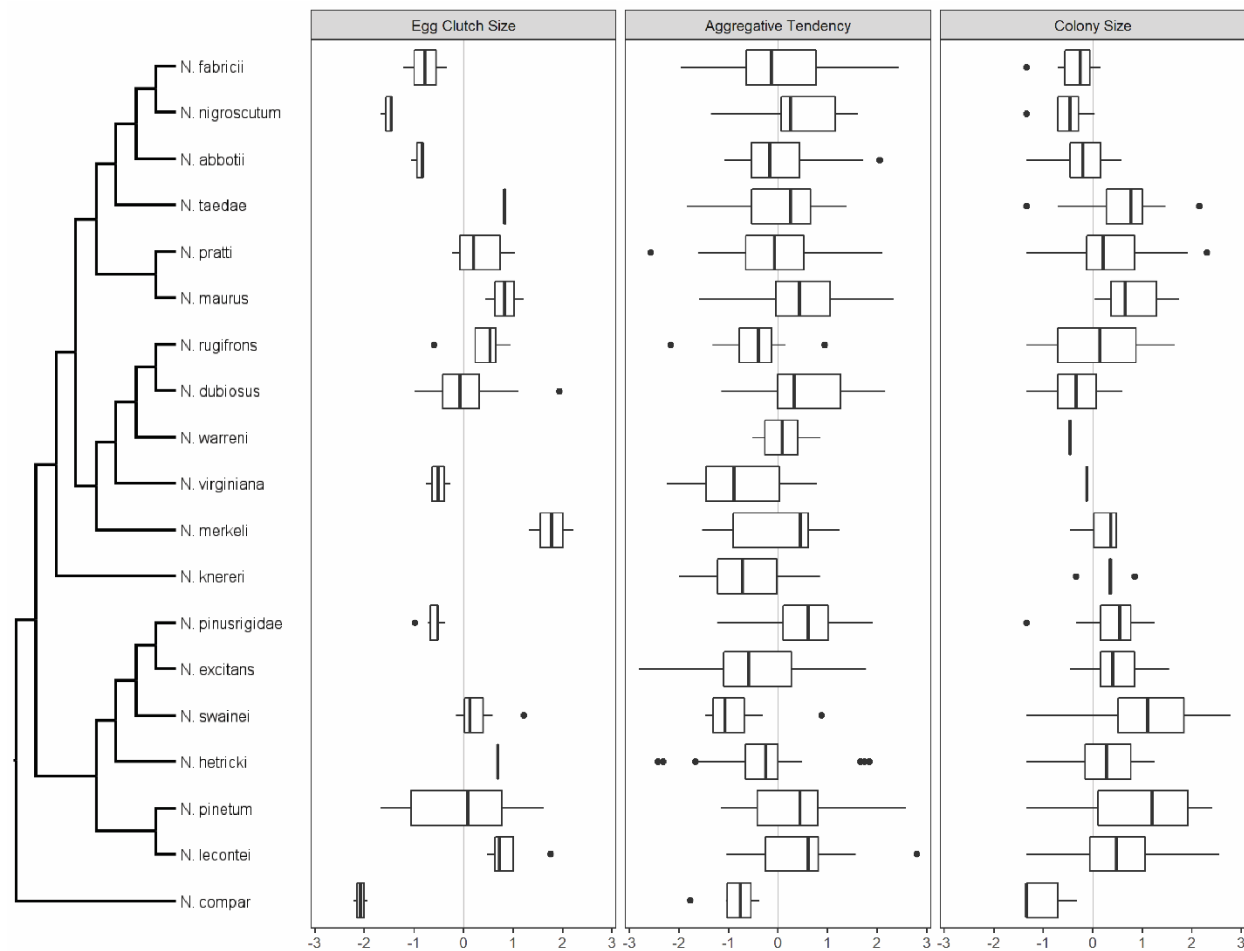
Phylogenetic signal in group-size traits

224   Overall, we found strong evidence of phylogenetic signal for larval colony size, weak evidence

225   for egg-clutch size, and no evidence for larval aggregative tendency. For larval colony size, the mean

226   estimated λ across 15,000 trees was close to 1 (λ=0.96 ± 0.05 s.d.; Fig. 2). Moreover, while 58% of the

227   trees rejected the model in which λ=0 (at α=0.05), only 0.03% of the trees rejected the λ=1 model.

228   Although λ estimates for egg-clutch size were also high (mean estimated λ=0.83 ± 0.37 s.d.; Fig. 2), none

229   of the trees rejected any of the fixed-λ models (0% for both λ=1 and λ=0). This difference may be

230   attributable, in part, to the smaller sample size for egg-clutch size (N = 16). In support of this

231   interpretation, we also failed to reject fixed-λ models when we repeated the larval colony size analysis

232   using only those taxa present in the female clutch size analysis (estimated λ=0.84 ± 0.10 s.d. , 0% reject

233   λ=1, 0.17% reject λ=0). In contrast, aggregative tendency consistently rejected models in which λ=1

234   (100%), but not λ=0 (0%), regardless of sample size. For this trait, the mean estimated λ was 0 (± 0.00

235   s.d.; Fig. 2).

236   **Figure 2. Egg-clutch size has a weak phylogenetic signal; aggregative tendency has no**

237   **evidence of a phylogenetic signal; and larval colony size has a strong phylogenetic**

238   **signal.** Histograms of estimated lambda values across 15,000 trees sampled from the

239   posterior distribution of a Bayesian analysis (Linnen and Farrell 2008a; Linnen and

240   Farrell 2010) for the three traits of interest: egg-clutch size, aggregative tendency, and

241   colony size. Noted on each histogram is the percentage of trees that rejected the fixed

242   lambda models of λ=0 and λ=1.


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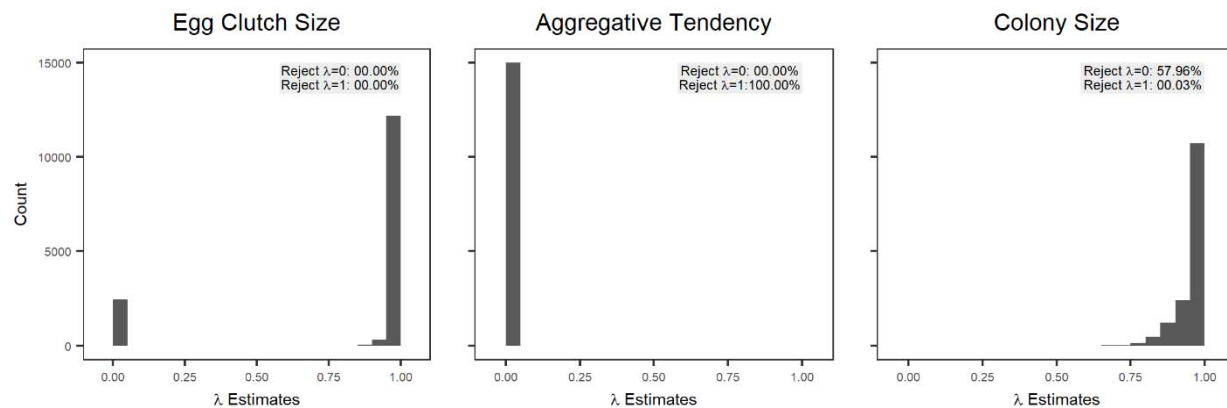
244   **Relationships between group-size traits**

245   Regardless of whether or not we corrected for phylogeny, we found no evidence of a correlation

246   between egg-clutch size and aggregative tendency (non-phylogenetic: r = 0.0255, p = 0.5547, Fig. 3A;

247   phylogenetic: estimated r = -0.0733 ± 0.0495 s.d., 0% reject null, Fig. 3D). Likewise, we found no

248   evidence that larval aggregative tendency predicted larval colony size across species (non-phylogenetic:

249     $F_{1,17}$ = 0.0216, R^2 = 0.0013, p = 0.8850, Fig. 3C; phylogenetic: estimated R^2 =0.0870 ± 0.0289, 0.005%

250   reject null, Fig. 3F). In contrast, our regression analyses consistently indicated a relationship between

251   egg-clutch size and larval colony size (non-phylogenetic: $F_{1,14}$ = 13.8751, R^2 = 0.4978, p = 0.0023, Fig. 3B;

252   phylogenetic: estimated R^2 =0.4997 ± 0.0356, 100% reject null, Fig. 3E). Importantly, these relationships

253   were in the predicted direction: larger egg-clutches consistently produced larger larval groups.

254   **Figure 3. Larval aggregative tendency is not correlated with egg-clutch size and does**

255   **not influence larval colony size; however, egg-clutch size predicts larval colony size.**

256   Panels **A-C** represent analyses lacking phylogenetic correction: egg-clutch size vs. larval

257   aggregative tendency (correlation test, A), egg-clutch size vs. larval colony size

258   (regression analysis, B), and aggregative tendency vs. larval colony size (regression

259   analysis, C). Panels D-F are histograms of phylogenetically corrected correlation

260   coefficients between traits estimated for 15,000 trees from the posterior distribution of

261   a Bayesian analysis (Linnen and Farrell 2008a; Linnen and Farrell 2010): egg-clutch size

262   vs. larval aggregative tendency (correlation test, D), egg-clutch size vs. larval colony size

263   (regression analysis, E), and aggregative tendency vs. larval colony size (regression

264   analysis, F).

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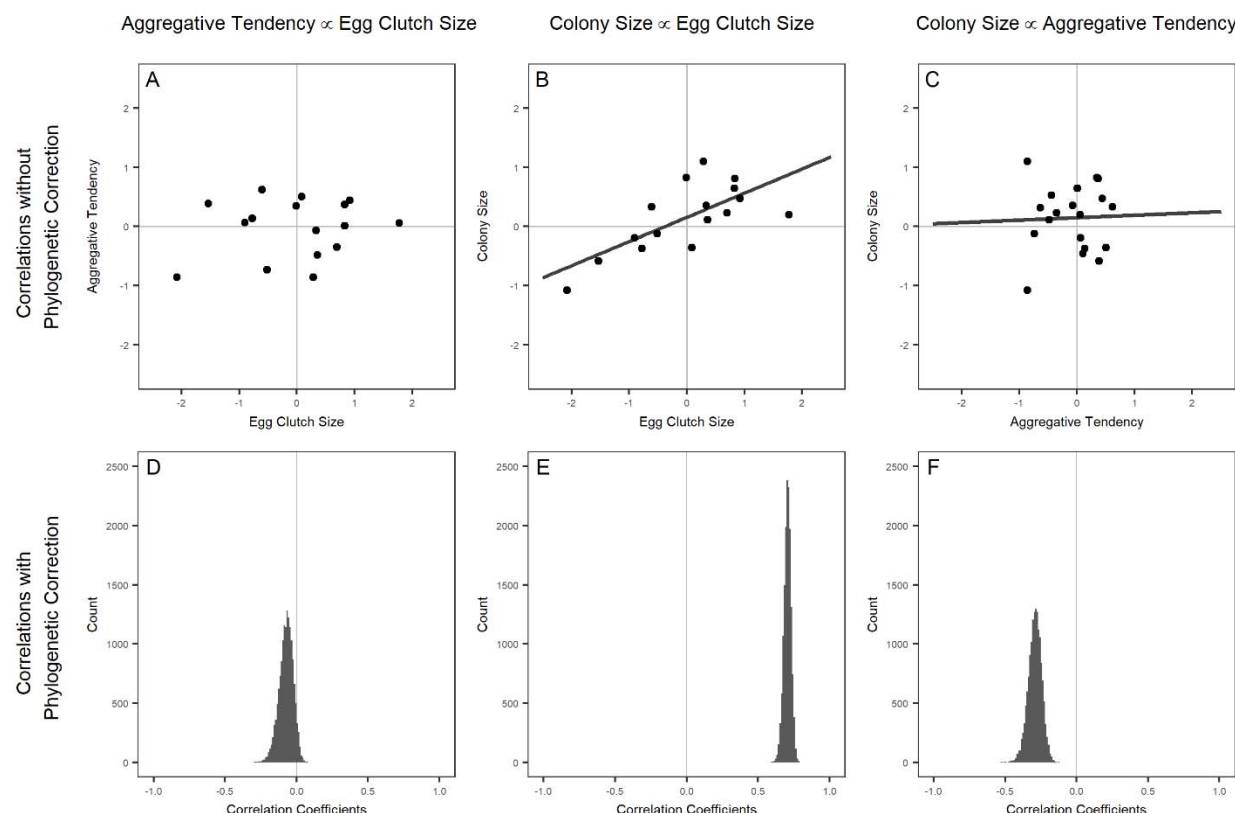
266   Of the eight possible statistical models incorporating some combination of the explanatory

267   traits, egg-clutch size and aggregative tendency, and their interaction; the best fitting model with the

268   lowest AIC_C score was egg-clutch size alone (AIC_C = 23.77864; p = 0.0023 compared to a null model). The

269   next most likely model used egg-clutch size and the interaction between egg-clutch size and aggregative

270   tendency as explanatory variables (AIC_C = 25.92652, p = 0.0062 compared to a null model). The third

271   most likely model used egg-clutch size and aggregative tendency as explanatory variables (AIC_C =

272   27.41347, p = 0.0114 compared to a null model). All other statistical models had Δ AIC_C scores greater

273   than 5. The p-values for comparisons to a null model, AIC_C scores, Δ AIC_C scores, Aikaike weights and

274   evidence ratios for all statistical models are available in Table 1.

275   **Table 1. Egg-clutch size is a better predictor of larval colony size than larval**

276   **aggregative tendency.** Results for non-phylogenetically corrected model comparisons.

277   In the "Model" column, "C" refers to "colony size", "E" to "egg-clutch size", and "A" to

278   "aggregative tendency". The columns contain the p-values for each model compared to

279   the null model (colony size alone), AICc are the Akaike information critera corrected for

280   sample size, ΔAICc are the differences between AIC scores from the best model (C ~ E),

281   w_i are the Akaike weights for each model, and ER_i are the evidence ratios of each model

282   compared to the best model (C ~ E).

Model	p-value	AICc	ΔAICc	w_i	ER_i
C ~ E + A + E*A	0.0196	30.097	6.318	0.027	23.552
C ~ E + A	0.0114	27.413	3.635	0.103	6.156
C ~ E + E*A	0.0062	25.927	2.148	0.216	2.927
C ~ A + E*A	0.4044	36.205	12.427	0.001	499.326
C ~ E*A	0.1707	32.575	8.797	0.008	81.315
C ~ E	0.0023	23.779	0	0.631	1
C ~ A	0.6593	34.567	10.789	0.003	220.151
C	.	31.721	7.942	0.012	53.036

283

284 **Discussion**

285   Comparative data across diverse systems suggest that antagonistic coevolution stemming from

286 evolutionary conflicts can fuel rapid diversification of traits and species (Crespi and Nosil 2013; Carmona

287 et al. 2015; Queller and Strassmann 2018). Such dynamics have been confirmed in the context of parent-

288 offspring conflict in organisms providing substantial parental care (Briskie et al. 1994; Lloyd and Martin

289 2003). Although conflicts are also expected even in the absence of parental care (Godfray and Parker

290 1987; Parker and Mock 1987; Godfray and Parkre 1992; Roitbeerg and Mangel 1993; Rosenheim 1993;

291   Sjerps and Haccou 1994), comparative studies examining such systems are lacking (but see Mayhew

292   1998). In this study, we have taken advantage of a genus of herbivorous insects with well-documented

293   interspecific variation in traits that mediate group size to ask whether evolutionary dynamics are

294   consistent with parent-offspring conflict. While we find extensive interspecific variation in all three traits

295   and some decoupling from phylogeny, there is no evidence of coevolution between adult female

296   oviposition behavior and larval grouping behavior. Furthermore, while larvae have the physical capacity

297   to disperse (Benjamin 1955; Smirnoff 1960; Codella and Raffa 1995), the close correspondence between

298   egg-clutch size and larval colony size suggests that variable aggregative behavior in the larvae does not

299   substantially alter the initial clutch size. A discussion of possible limitations of our phenotypic datasets

300   can be found in the supplemental materials. Here, we consider possible biological explanations for the

301   apparent lack of strong parent-offspring conflict over group size and propose alternative explanations

302   for interspecific variation in group-living traits.

303   One potential explanation for the apparent lack of correlated evolution between female clutch-

304   size and larval grouping behavior is that populations often lack heritable variation in grouping traits that

305   mediate conflicts over group size. However, this explanation seems unlikely because we have detected

306   considerable interspecific variation in both oviposition traits and larval aggregative tendency, even when

307   measured under uniform laboratory conditions (Fig. 1). Although non-genetic sources (e.g., plasticity)

308   may explain some of this variation, experimental crosses have confirmed that some of this behavioral

309   variation is attributable to genetic differences between populations and species (Knerer and Atwood

310   1972; Bendall et al 2017). That said, even with genetic variation for adult and larval behaviors,

311   physiological limits on some traits could constrain trait evolution in response to parent-offspring conflict

312   over group size. For example, sawfly females typically emerge from cocoons with their full complements

313   of eggs (Coppel and Benjamin 1965), placing an upper limit on maximum clutch size. This upper limit

314   could prevent egg-clutch sizes from becoming large enough to generate strong selection on dispersal

315   tendencies, thereby limiting the potential for repeated bouts of reciprocal evolutionary change.

316   Assuming that populations could respond to selection stemming from parent-offspring conflict,

317   another explanation for a lack of correlated evolution is that conflict is too weak or idiosyncratic to

318   produce such a pattern. For example, many theoretical studies on group-size conflict assume that larval

319   fitness is optimized at a group size of n = 1 (Parker and Mock 1987; Godfray and Parker 1992; Roitberg

320   and Mangel 1993; but see Godfray and Ives 1988), ignoring possible advantages to larval grouping (e.g.,

321   an Allee effect (Courchamp et al. 1999)). By contrast, potential benefits of group-living are well

322   documented (Seymour 1974; Bertram 1978; Young and Moffett 1979; Tsubaki and Shiotsu 1982; Pulliam

323   and Caraco 1984; Joos et al. 1988; Stamp and Bowers 1990; Codella and Raffa 1993; Klok and Chown

324   1999; Despland and Le Huu 2007; Fletcher 2009; McClure et al. 2011a; McClure et al. 2011b; McClure et

325   al. 2013; Campbell and Stastny 2015). In *Neodiprion*, it has been hypothesized that group-living helps

326   larvae overcome host defenses (Ghent 1960, but see Kalin and Knerer 1977). If benefits of group-living

327   consistently outweigh costs in *Neodiprion*, parent-offspring conflict over group size could be minimal.

328   Overall, our data suggest that parent-offspring conflict is not the predominant force shaping

329   interspecific variation in female clutch size and larval grouping behavior. Another potential explanation

330   for among-species variation in these traits is that it accumulated via genetic drift as species diverged

331   from common ancestors. Under this scenario, however, we would expect a strong phylogenetic signal

332   for grouping traits. By contrast, our data indicate that traits are at least partially (female clutch-size) or

333   completely (larval aggregative tendency) decoupled from phylogeny. Therefore, we now consider other

334   possible selection pressures that may shape intra- and interspecific variation in these traits. Ultimately,

335   testing these hypotheses will require a combination of experiments that verify targets and agents of

336   selection and comparative analyses that evaluate the contribution of different selective mechanisms to

337   genus-wide variation.

338   For an adult female, optimal clutch size depends on the distribution and quality of available host

339   plants, the physiological condition of the female, and the relationship between clutch size and offspring

340   survival (Skinner 1985; Mangel 1987; Tostowaryk 1972). Variation in any of these factors could favor

341   different oviposition strategies in different species. For example, *Neodiprion* species vary in their

342   preferred oviposition hosts (Knerer and Atwood 1972; Knerer and Atwood 1973; Linnen and Farrell

343   2010; Bendall et al. 2017). If preferred hosts differ in their patchiness, different oviposition strategies

344   may be favored. Interspecific variation in clutch size may also arise if the relationship between clutch

345   size and larval survival varies among species. For example, some *Neodiprion* species have aposematic

346   coloration (Tostwaryk 1972; Linnen et al. 2018). When aposematic signals are used, group-living can

347   enhance the efficacy of those signals (Gamberale and Tullberg 1998; Hatle and Salazar 2001; Riipi et al.

348   2001). Anecdotally, *Neodiprion* with brighter coloration (e.g., *Neodiprion lecontei*) tend to have larger

349   colony sizes than more cryptically colored species (e.g., *N. compar*). However, additional comparative

350   work is needed to determine whether group-living correlates with conspicuousness in *Neodiprion*.

351   For larvae, although aggregative tendency does not appear to impact larval group size, it may

352   nevertheless play an important role in larval survival. By controlling the density of larvae within groups,

353   variation in aggregative tendency can impact disease transmission rates, microclimate, and host physical

354   and chemical defenses. First, because disease transmission occurs primarily through physical contact,

355   reduced disease transmission is one possible advantage of having a diffuse larval group (i.e., low

356   aggregative tendency). Notably, nucleopolyhedrovirus and other infectious diseases can be a major

357   source of mortality for sawfly larvae and gregarious larvae of ecologically similar species (Bird 1955;

358   Mohamed et al 1985; Young and Yearian 1987; Hochbereg 1991; Fletcher 2009). Second, through

359   managing the larval group's density, larval aggregative tendency could also be a mechanism through

360   which group-living species optimize their micro-environment (Seymour 1974; Joos et al. 1988; Codella

361   and Raffa 1993; Klok and Chown 1999; Fletcher 2009; McClure et al. 2011a). For example, compared to

362   warm and wet environments, cold or dry environments may favor tighter clustering of larval groups.

363   Third, variation in pine needle morphology and defensive chemistry among pine species may favor

364   different larval densities. When feeding in groups, sawfly larvae form a characteristic "ring" around a

365   needle near the tip and, as a group, consume the needle tissue down to the fascicle (Ghent 1960).

366   However, the ability to form a stable feeding ring may decrease on thin- and long-needled hosts.

367   Reduced aggregative tendency may prevent larvae from overloading thin needles unable to bear their

368   weight. By contrast, thick and resinous needles may favor increased aggregative tendency to ensure a

369   sufficient number of larvae to establish feeding sites and distribute host resins (Ghent 1960).

370

371   **Conclusions**

372   Comparative analysis is a powerful tool for testing and honing intuitions derived from

373   evolutionary theory. For example, although theoretical work suggests that small, gregarious broods of

374   parasitoids are evolutionary unstable and transitions from solitary to gregarious states should be rare

375   (Godfray 1987), clutch-size data from parasitoid wasps suggests that such transitions have occurred

376   many times independently (Mayhew 1998). Analogously, although theory demonstrates the potential

377   for parent-offspring conflict over group size in plant-feeding insects (Roitberg and Mangel 1993), our

378   data suggest that this conflict is not sufficiently strong or consistent to drive correlated evolution of

379   adult and larval grouping traits in pine sawflies. To be clear, these findings do not rule out parent-

380   offspring conflict entirely. Rather, they suggest that when parental care is minimal, parent-offspring

381   conflict is not a major driver of long-term patterns of phenotypic change. Although these trends are

382   intriguing, more comparative data are needed to evaluate whether some forms of conflict are more

383   likely than others to leave a detectable phenotypic footprint in phylogenetic comparative analyses.

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606   **Figures and Tables**

607   **Figure 1. *Neodiprion* species vary in multiple group-size traits.** Phylogenetic relationships for 19 eastern

608   North American *Neodiprion* are from (Linnen and Farrell 2008a; Linnen and Farrell 2010). Box plots

609   depict intraspecific variation for egg-clutch size, larval aggregative tendency, and larval colony size. For

610   all three traits, there is significant interspecific variation (ANOVAs, P<0.001).

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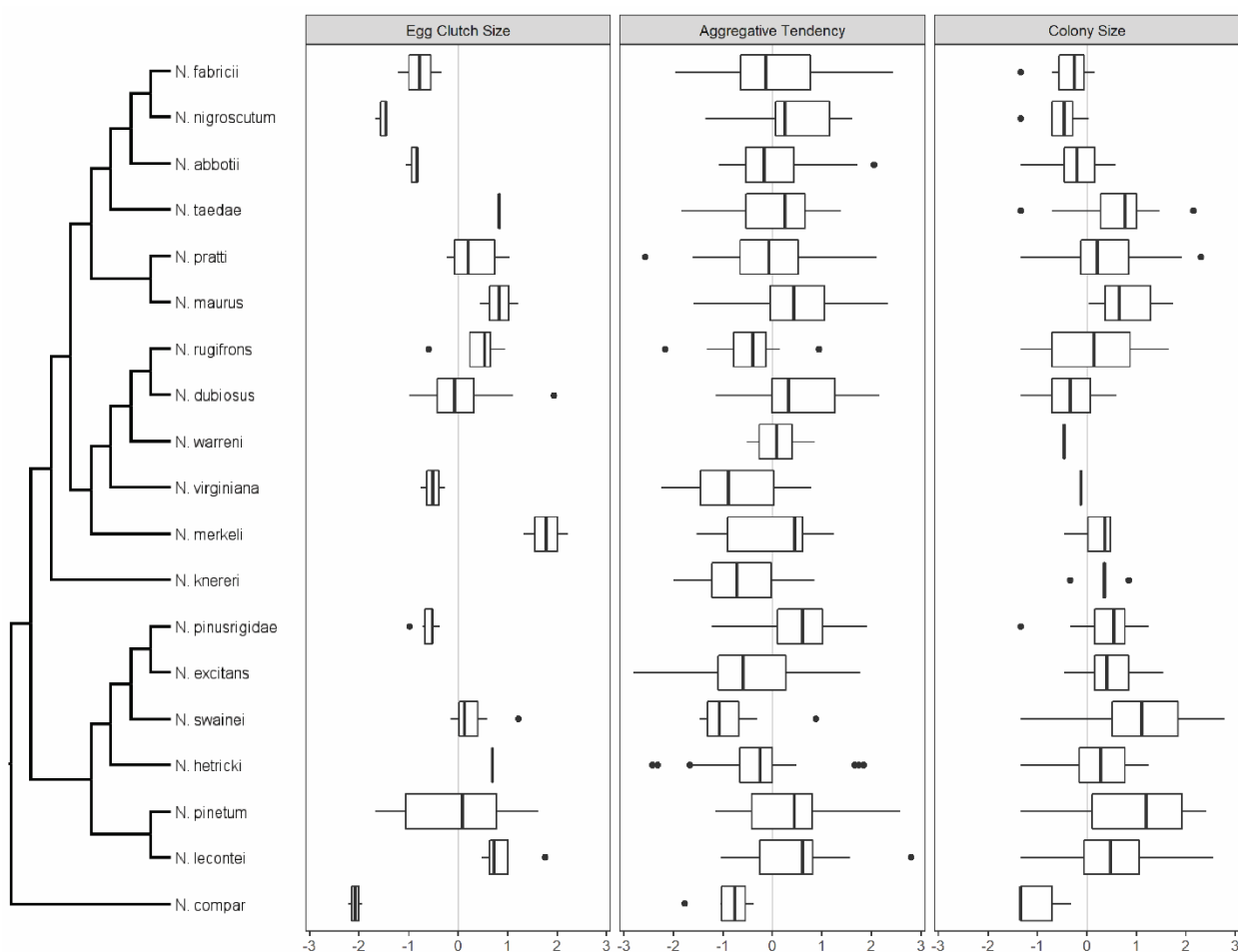
613   **Figure 2. Egg-clutch size has a weak phylogenetic signal; aggregative tendency has no evidence of a**

614   **phylogenetic signal; and larval colony size has a strong phylogenetic signal.** Histograms of estimated

615   lambda values across 15,000 trees sampled from the posterior distribution of a Bayesian analysis

616   (Linnen and Farrell 2008a; Linnen and Farrell 2010) for the three traits of interest: egg-clutch size,

617   aggregative tendency, and colony size. Noted on each histogram is the percentage of trees that rejected

618   the fixed lambda models of λ=0 and λ=1.

619

620

621   **Figure 3. Larval aggregative tendency is not correlated with egg-clutch size and does not influence**

622   **larval colony size; however, egg-clutch size predicts larval colony size.** Panels A-C represent analyses

623   lacking phylogenetic correction: egg-clutch size vs. larval aggregative tendency (correlation test, A), egg-

624   clutch size vs. larval colony size (regression analysis, B), and aggregative tendency vs. larval colony size
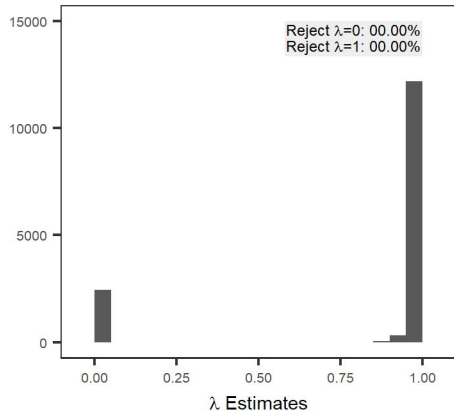
625   (regression analysis, C). Panels D-F are histograms of phylogenetically corrected correlation coefficients

626   between traits estimated for 15,000 trees from the posterior distribution of a Bayesian analysis (Linnen

627   and Farrell 2008a; Linnen and Farrell 2010): egg-clutch size vs. larval aggregative tendency (correlation

628   test, D), egg-clutch size vs. larval colony size (regression analysis, E), and aggregative tendency vs. larval

629   colony size (regression analysis, F).

630

631

632   **Table 1. Egg-clutch size is a better predictor of larval colony size than larval aggregative tendency.**

633   Results for non-phylogenetically corrected model comparisons. In the "Model" column, "C" refers to

634   "colony size", "E" to "egg-clutch size", and "A" to "aggregative tendency". The columns contain the p-

635   values for each model compared to the null model (colony size alone), AICc are the Akaike information

636   critera corrected for sample size, ΔAICc are the differences between AIC scores from the best model (C ~

637   E), w_i are the Akaike weights for each model, and ER_i are the evidence ratios of each model compared to

638   the best model (C ~ E).

Model	p-value	AICc	ΔAICc	w_i	ER_i
C ~ E + A + E*A	0.0196	30.097	6.318	0.027	23.552
C ~ E + A	0.0114	27.413	3.635	0.103	6.156
C ~ E + E*A	0.0062	25.927	2.148	0.216	2.927
C ~ A + E*A	0.4044	36.205	12.427	0.001	499.326
C ~ E*A	0.1707	32.575	8.797	0.008	81.315
C ~ E	0.0023	23.779	0	0.631	1
C ~ A	0.6593	34.567	10.789	0.003	220.151
C	.	31.721	7.942	0.012	53.036

639

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