

# 1 **Parasite dispersal influences introgression rate**

2 Jorge Doña<sup>1,2</sup>, Andrew D. Sweet<sup>2,3</sup>, and Kevin P. Johnson<sup>2</sup>

3 <sup>1</sup>AllGenetics & Biology SL, Edificio CICA, Campus de Elviña s/n, 15008 A Coruña, Spain.

4 <sup>2</sup>Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-

5 Champaign, 1816S. Oak St., Champaign, Illinois 61820, USA.

6 <sup>3</sup>Department of Entomology, Purdue University, 901 W. State St., West

7 Lafayette, Indiana 47907, USA.

8

9

10

11

12

13

14

15

16

17 Dispersal is a central process in biology with implications at multiple scales of organization<sup>1,2,3,4</sup>.  
18 Organisms vary in their dispersal abilities, and these differences can have important biological  
19 consequences, such as impacting the likelihood of hybridization events<sup>5</sup>. However, the factors  
20 shaping the frequency of hybridization are still poorly understood, and therefore how dispersal  
21 ability affects the opportunities for hybridization is still unknown. Here, using the ecological  
22 replicate system of dove wing and body lice (Insecta: Phthiraptera)<sup>6</sup>, we show that species with  
23 higher dispersal abilities exhibited increased genomic signatures of introgression. Specifically,  
24 we found a higher proportion of introgressed genomic reads and more reticulated phylogenetic  
25 networks in wing lice, the louse group with higher dispersal abilities. Our results illustrate how  
26 differences in dispersal ability can drive differences in the extent of introgression through  
27 hybridization. The results from this study represent an important step for understanding the  
28 factors driving hybridization. We expect our approach will stimulate future studies on the  
29 ecological factors shaping hybridization to further understand this important process.

30

31           Dispersal is the permanent movement of organisms away from their place of origin. It is a  
32 fundamental process in biology with significant implications at multiple scales of  
33 organization<sup>1,2,3,4</sup>, including the reproduction of individuals, the composition of populations and  
34 communities, and the geographical distribution of species<sup>1,7</sup>.

35           Organisms differ in their dispersal abilities, and these differences have an impact on their  
36 biology, such as on the distributional range of a species or gene flow between populations<sup>5</sup>. For  
37 example, organisms with lower dispersal abilities tend to have smaller distributional ranges and  
38 populations that are genetically more structured<sup>5,8,9</sup>.

39           Dispersal ability might also affect the opportunities for hybridization between species  
40 because the rates at which individuals encounter different species are likely to be higher in  
41 organisms with higher dispersal capabilities. Indeed, recent evidence supports this prediction by  
42 demonstrating that range expansion is associated with the extent of introgression<sup>10,11</sup>. Similarly,  
43 dispersal differences explain more than 30% of the variation in the width of hybrid zones across  
44 animals<sup>12</sup>. However, overall the factors influencing hybridization events are poorly known<sup>13</sup>,  
45 and, in particular, the influence of dispersal ability on the rate of hybridization remains  
46 understudied.

47           Comparisons of the effect of dispersal on hybridization should ideally hold constant most  
48 factors other than dispersal. The ecological replicate system of wing and body lice (Insecta:  
49 Phthiraptera) of pigeons and doves (Aves: Columbidae) has proven to be an ideal system for  
50 comparing the impact of dispersal differences on other aspects of biology, such as population  
51 structure and codivergence<sup>6,8,14,15,16</sup>. Both of these two lineages of feather lice occur across the  
52 diversity of pigeons and doves and have the same basic life history and diet, but they  
53 significantly differ in their dispersal ability<sup>17,18,19</sup>. Both wing and body lice disperse vertically

54 between parents and offspring in the nest. However, wing lice can also attach to and hitchhike on  
55 hippoboscid flies to disperse “phoretically” between host individuals or host species<sup>17,18,19</sup>.  
56 Indeed, this additional dispersal mechanism profoundly influences their degree of  
57 population structure and cophylogenetic history<sup>8,14,16,20</sup>. In addition, wing lice have a higher rate  
58 of host-switching<sup>6,14,15</sup> (i.e., successful colonization of new host species) and of straggling<sup>21</sup>  
59 (i.e., dispersal to new host species without reproduction on that new host).

60 To compare differences in the extent of introgression between wing and body lice, we  
61 used whole-genome data from 71 louse individuals belonging to five taxa of wing lice  
62 (*Columbicola*) and seven taxa of body lice (*Physoconelloides*) occurring across the same host  
63 species. We predicted that wing lice, which have higher dispersal abilities and thus higher odds  
64 of encountering individuals of a different louse species on the same host, should show more  
65 extensive evidence of introgression (Fig. 1).

66 We used two different approaches to quantify the differences in introgression between  
67 louse genera. First, in individual louse genomes, we quantified the genomic contributions from  
68 different closely related louse species of the same genus<sup>22</sup>. Second, we quantified introgression at  
69 the species level, accounting for incomplete lineage sorting (ILS) by inferring phylogenetic  
70 networks using a maximum pseudo-likelihood framework<sup>23,24,25</sup>.

71 Both approaches revealed highly concordant results; higher levels of introgression among  
72 species of wing lice compared to body lice. In particular, using a read-mapping based method,  
73 the genomic signature of introgression was significantly higher in wing louse species than in  
74 body louse species (GLM with the mean values of the simulations;  $F = 21.0705$ ,  $df = 69$ ,  $P =$   
75  $2.367 \times 10^{-5}$ ; Fig. 2, Supplementary Table S1, Figs. S1-S12).

76           Secondly, in a phylogenetic network framework, the optimal networks of wing lice were  
77 more reticulated than those of body lice even though the number of taxa included in the networks  
78 was lower (seven reticulations in *Columbicola* vs. four in *Physconelloides*, Fig. 3). Accordingly,  
79 the number of reticulations given the number of potential combinations was significantly higher  
80 ( $\chi^2 = 3.8132$ ;  $df=1$ ;  $P= 0.03$ ). Also, the specific lineages involved in the reticulations were  
81 generally congruent with signatures of introgression from the read-mapping based approach (Fig.  
82 S1-S12).

83           Taken together, evidence from wing and body louse genomes suggests that differences in  
84 dispersal ability drive differences in the extent of introgression in this system of ecological  
85 replicate parasites. This work is among the first studies of introgression in a host-symbiont  
86 system<sup>26</sup>. Notably, recent studies have found that straggling and host switching are relatively  
87 common processes in host-symbiont systems<sup>27,28,29,30</sup>. Our study suggests that in a  
88 straggling/host-switching scenario, hybridization can provide further variation with important  
89 eco-evolutionary consequences<sup>31</sup>. Overall, the results from this study represent a significant step  
90 towards understanding the factors driving hybridization, because most previous studies focus on  
91 the presence/absence of hybridization and the evolutionary consequences of hybridization  
92 events<sup>13,32</sup>. Further research is needed to understand the factors shaping the frequency of  
93 hybridization and how these factors influence eco-evolutionary dynamics.

94

## 95 **Methods**

### 96 Data

97 We studied whole genome data from 71 louse individuals belonging to five and seven taxa  
98 of *Columbicola* and *Physconelloides*, respectively (Supplementary Table S2). Data were  
99 available from previous studies<sup>16,33,34</sup> and represent all described New World ground-dove wing  
100 and body louse species, most host species in this group, and sampling across multiple  
101 biogeographic areas within species<sup>16</sup> (Supplementary Table S2). Illumina genome sequence data  
102 pre-processing included several steps<sup>16</sup>. First, we discarded duplicate read pairs using the  
103 *fastqSplitDups* script ([https://github.com/McIntyre-](https://github.com/McIntyre-Lab/mcscriptand)  
104 [Lab/mcscriptand](https://github.com/McIntyre-Lab/mcscriptand) <https://github.com/McIntyre-Lab/mcscriptand>). We then eliminated the Illumina  
105 sequencing adapters with *Fastx\_clipper* v0.014 from the FASTX-Toolkit  
106 ([http://hannonlab.cshl.edu/fastx\\_toolkit](http://hannonlab.cshl.edu/fastx_toolkit)). Also, we removed the first 5 nt from the 5' ends of  
107 reads using *Fastx\_trimmer* v0.014 and trimmed bases from the 3' ends of reads until reaching a  
108 base with a phred score  $\geq 28$  using *Fastq\_quality\_trimmer* v0.014. Finally, we removed any reads  
109 less than 75 nt and analyzed the cleaned libraries with *Fastqc* v0.11.5 to check for additional  
110 errors. We assembled nuclear loci in aTRAM following previous studies<sup>16,33,34,35</sup>. In particular,  
111 we mapped modest coverage (25-60X), multiplexed genomic data to reference loci from a  
112 closely related taxon. For our reference set of nuclear loci for wing lice, we used 1,039 exons  
113 of *Columbicola drowni* generated in a previous study<sup>33</sup> (raw data: SRR3161922). This data set  
114 was assembled de novo<sup>35</sup> using orthologous protein-coding genes from the human body louse  
115 genome (*Pediculus humanus humanus*<sup>36</sup>) as a set of target sequences. We mapped our newly  
116 generated *Columbicola* reads and the reads obtained from GenBank to the *C. drowni* references  
117 using *Bowtie2*<sup>37</sup>. For body lice, we obtained nuclear data using the same pipeline and software

118 parameters, except that we used 1,095 loci from *P. emersoni* as the reference for mapping. To  
119 generate the input ultrametric gene trees for Phylonet v3.6.8<sup>23,24,25</sup>, we first aligned each nuclear  
120 locus in MAFFT<sup>38</sup>(--auto) and removed columns with only ambiguous sequences (“N”). Then,  
121 we estimated gene trees in RAxML v8.1.3<sup>39</sup> with a GTR +  $\Gamma$  substitution model for each gene  
122 alignment. Finally, we made trees ultrametric using the nmls method in the *force.ultrametric*  
123 function within the “phytools” R package<sup>40</sup>.

#### 124 Quantifying introgression

125 We used two different approaches to quantify differences in the extent of introgression between  
126 the two louse genera. First, we used sppIDer<sup>22</sup> to quantify the genomic contributions of different  
127 louse species in an individual louse genome. We built our reference for each genus using all the  
128 nuclear loci from a single individual per species. For the reference, we selected those individuals  
129 for which we assembled the highest number of loci. Finally, we estimated the extent of  
130 introgression as the sum of the mean coverages of reads mapped from all the species excluding  
131 the focal louse species, divided by the mean coverage of the focal louse species. Second, we  
132 quantified introgression at the species level, while accounting for ILS, using a maximum pseudo-  
133 likelihood framework with PhyloNet 3.6.1<sup>23,24,25</sup>. We trimmed the unrooted gene trees to the  
134 same individuals used as reference taxa in sppIDer, and performed ten independent analyses with  
135 a differing maximum number of reticulation nodes (i.e., from zero to ten). We conducted ten  
136 runs per analysis. We then selected the optimal network for each genus based on AIC values.

#### 137 Analyses

138 We compared the sppIDer results using generalized linear models (GLMs). We used a Gaussian  
139 distribution of errors and an identity link function. We performed one GLM for each simulation

140 iteration using the *glm* function of the “stats” R package<sup>41</sup>. The extent of introgression for each  
141 louse genus was the dependent variable, the genus identity was the independent variable, and we  
142 accounted for the introgression differences between louse species including louse identity as a  
143 fixed factor. We confirmed assumptions underlying GLMs by testing the normality of regression  
144 residuals for normality against a Q-Q plot. We also considered the possibility that some of the  
145 reads mapping to other species were technical contaminations, i.e., due to index-  
146 swapping<sup>42,43,44,45</sup>. To account for possible contaminants, we wrote a simulation in R that  
147 randomly subtracted 9% from the mean coverage value of a particular sample (i.e., we subtracted  
148 a random proportion of the mean coverage value for each species until reaching 9 %). We ran  
149 100 iterations of the simulation and ran a GLM for each iteration (Table S1). Finally, we used  
150 the  $\chi^2$  test to compare the number of species in pairwise comparisons of each genus with the  
151 number of reticulations found in each optimal phylogenetic network.

152

## 153 **References**

- 154 1 Clobert J, Danchin E, Dhondt AA, Nichols J D. *Dispersal*. Oxford Univ. Press, 2001.
- 155 2 Nathan R. The challenges of studying dispersal. *Trends in Ecology & Evolution* 2001; **16**: 481–  
156 483.
- 157 3 Matthysen E. Multicausality of dispersal: a review. In: *Dispersal Ecology and Evolution*.  
158 Oxford University Press, 2012, pp 3–18.
- 159 4 Barton NH. The genetic consequences of dispersal. In: *Animal Dispersal*. Springer  
160 Netherlands, 1992, pp 37–59.



- 161 5 Bohonak AJ. Dispersal Gene Flow, and Population Structure. *The Quarterly Review of Biology*  
162 1999; **74**: 21–45.
- 163 6 Clayton DH, Bush SE, Johnson KP. *Coevolution of Life on Hosts*. University of Chicago Press,  
164 2016 doi:10.7208/chicago/9780226302300.001.0001.
- 165 7 Clobert J, Baguette M, Benton TG, Bullock JM (eds.). *Dispersal Ecology and Evolution*.  
166 Oxford University Press, 2012 doi:10.1093/acprof:oso/9780199608898.001.0001.
- 167 8 DiBlasi E, Johnson KP, Stringham SA, Hansen AN, Beach AB, Clayton DH *et al.* Phoretic  
168 dispersal influences parasite population genetic structure. *Molecular Ecology* 2018; **27**:  
169 2770–2779.
- 170 9 Dawson MN, Hays CG, Grosberg RK, Raimondi PT. Dispersal potential and population  
171 genetic structure in the marine intertidal of the eastern North Pacific. *Ecological*  
172 *Monographs* 2014; **84**: 435–456.
- 173 10 Nussberger B, Currat M, Quilodran CS, Ponta N, Keller LF. Range expansion as an  
174 explanation for introgression in European wildcats. *Biological Conservation* 2018; **218**:  
175 49–56.
- 176 11 Currat M, Ruedi M, Petit RJ, Excoffier L. The hidden side of invasions: massive introgression  
177 by local genes. *Evolution* 2008; **62**: 1908–20.
- 178 12 McEntee JP, Burleigh JG, Singhal S. Dispersal predicts hybrid zone widths across animal  
179 diversity: Implications for species borders under incomplete reproductive isolation.  
180 *BioRxiv* 2018. doi:10.1101/472506.

- 181 13 Arnold ML. *Divergence with Genetic Exchange*. Oxford University Press, 2015  
182 doi:10.1093/acprof:oso/9780198726029.001.0001.
- 183 14 Clayton DH, Johnson KP. Linking coevolutionary history to ecological process: doves and  
184 lice. *Evolution* 2003; **57**: 2335–41.
- 185 15 Johnson KP, Clayton DH. Untangling coevolutionary history. *Syst Biol* 2004; **53**: 92–4.
- 186 16 Sweet AD, Johnson KP. The role of parasite dispersal in shaping a host-parasite system at  
187 multiple evolutionary scales. *Molecular Ecology* 2018. doi:10.1111/mec.14937.
- 188 17 Harbison CW, Bush SE, Malenke JR, Clayton DH. Comparative transmission dynamics of  
189 competing parasite species. *Ecology* 2008; **89**: 3186–3194.
- 190 18 Harbison CW, Jacobsen MV, Clayton DH. A hitchhiker’s guide to parasite transmission: The  
191 phoretic behaviour of feather lice. *International Journal for Parasitology* 2009; **39**: 569–  
192 575.
- 193 19 Bartlow AW, Villa SM, Thompson MW, Bush SE. Walk or ride? Phoretic behaviour of  
194 amblyceran and ischnoceran lice. *International Journal for Parasitology* 2016; **46**: 221–  
195 227.
- 196 20 Sweet AD, Chesser RT, Johnson KP. Comparative cophylogenetics of Australian phabine  
197 pigeons and doves (Aves: Columbidae) and their feather lice (Insecta: Phthiraptera).  
198 *International Journal for Parasitology* 2017; **47**: 347–356.
- 199 21 Whiteman NK, Santiago-Alarcon D, Johnson KP, Parker PG. Differences in straggling rates  
200 between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and  
201 cophylogenetic patterns. *International Journal for Parasitology* 2004; **34**: 1113–1119.

- 202 22 Langdon QK, Peris D, Kyle B, Hittinger CT. sppIDer: A Species Identification Tool to  
203 Investigate Hybrid Genomes with High-Throughput Sequencing. *Mol Biol Evol* 2018; **35**:  
204 2835–2849.
- 205 23 Than C, Ruths D, Nakhleh L. PhyloNet: a software package for analyzing and reconstructing  
206 reticulate evolutionary relationships. *BMC Bioinformatics* 2008; **9**: 322.
- 207 24 Wen D, Yu Y, Zhu J, Nakhleh L. Inferring Phylogenetic Networks Using PhyloNet. *Syst Biol*  
208 2018; **67**: 735–740.
- 209 25 Yu Y, Nakhleh L. A maximum pseudo-likelihood approach for phylogenetic networks. *BMC*  
210 *Genomics* 2015; **16 Suppl 10**: S10.
- 211 26 Detwiler JT, Criscione CD. An infectious topic in reticulate evolution: introgression and  
212 hybridization in animal parasites. *Genes (Basel)* 2010; **1**: 102–23.
- 213 27 Doña J, Serrano D, Mironov S, Montesinos-Navarro A, Jovani R. Unexpected bird-feather  
214 mite associations revealed by DNA metabarcoding uncovers a dynamic ecoevolutionary  
215 scenario. *Molecular Ecology* 2018. doi:10.1111/mec.14968.
- 216 28 Bourguignon T, Lo N, Dietrich C, Šobotník J, Sidek S, Roisin Y *et al.* Rampant Host  
217 Switching Shaped the Termite Gut Microbiome. *Current Biology* 2018; **28**: 649–654.e2.
- 218 29 Vienne DM de, Refrégier G, López-Villavicencio M, Tellier A, Hood ME, Giraud T.  
219 Cospeciation vs host-shift speciation: methods for testing evidence from natural  
220 associations and relation to coevolution. *New Phytologist* 2013; **198**: 347–385.

- 221 30 Nylin S, Agosta S, Bensch S, Boeger WA, Braga MP, Brooks DR *et al.* Embracing  
222 Colonizations: A New Paradigm for Species Association Dynamics. *Trends in Ecology &*  
223 *Evolution* 2018; **33**: 4–14.
- 224 31 Barton NH. The consequences of an introgression event. *Molecular Ecology* 2018; **27**: 4973–  
225 4975.
- 226 32 Folk RA, Soltis PS, Soltis DE, Guralnick R. New prospects in the detection and comparative  
227 analysis of hybridization in the tree of life. *Am J Bot* 2018; **105**: 364–375.
- 228 33 Boyd BM, Allen JM, Nguyen N, Sweet AD, Warnow T, Shapiro MD *et al.* Phylogenomics  
229 using Target-restricted Assembly Resolves Intra-generic Relationships of Parasitic Lice  
230 (Phthiraptera: Columbicola). *Systematic Biology* 2017; syx027.
- 231 34 Sweet AD, Boyd BM, Allen JM, Villa SM, Valim MP, Rivera-Parra JL *et al.* Integrating  
232 phylogenomic and population genomic patterns in avian lice provides a more complete  
233 picture of parasite evolution. *Evolution* 2017; **72**: 95–112.
- 234 35 Allen JM, Huang DI, Cronk QC, Johnson KP. aTRAM - automated target restricted assembly  
235 method: a fast method for assembling loci across divergent taxa from next-generation  
236 sequencing data. *BMC Bioinformatics* 2015; **16**. doi:10.1186/s12859-015-0515-2.
- 237 36 Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM *et al.* Genome sequences of  
238 the human body louse and its primary endosymbiont provide insights into the permanent  
239 parasitic lifestyle. *Proc Natl Acad Sci U S A* 2010; **107**: 12168–73.
- 240 37 Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2.. *Nat Methods* 2012; **9**:  
241 357–9.

- 242 38 Katoh K. MAFFT: a novel method for rapid multiple sequence alignment based on fast  
243 Fourier transform. *Nucleic Acids Research* 2002; **30**: 3059–3066.
- 244 39 Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with  
245 thousands of taxa and mixed models. *Bioinformatics* 2006; **22**: 2688–2690.
- 246 40 Revell LJ. phytools: an R package for phylogenetic comparative biology (and other things).  
247 *Methods in Ecology and Evolution* 2011; **3**: 217–223.
- 248 41 Team RC, others. R: A language and environment for statistical computing. 2013.
- 249 42 Carlsen T, Aas AB, Lindner D, Vrålstad T, Schumacher T, Kauserud H. Dont make a  
250 mista(g)ke: is tag switching an overlooked source of error in amplicon pyrosequencing  
251 studies?. *Fungal Ecology* 2012; **5**: 747–749.
- 252 43 Schnell IB, Bohmann K, Gilbert MT. Tag jumps illuminated—reducing sequence-to-sample  
253 misidentifications in metabarcoding studies. *Mol Ecol Resour* 2015; **15**: 1289–303.
- 254 44 Esling P, Lejzerowicz F, Pawlowski J. Accurate multiplexing and filtering for high-  
255 throughput amplicon-sequencing. *Nucleic Acids Res* 2015; **43**: 2513–24.
- 256 45 Sinha R, Stanley G, Gulati GS, Ezran C, Travaglini KJ, Wei E *et al*. Index Switching Causes  
257 Spreading-Of-Signal Among Multiplexed Samples In Illumina HiSeq 4000 DNA  
258 Sequencing. *BioRxiv* 2017. doi:10.1101/125724.

259  
260 **Additional information**

261 Table S1, S2 and, Figures S1-S12 are embedded into the supplementary\_material.html file.

262

263 **Acknowledgements**

264 This study was supported by NSF DEB-1239788 and DEB-1342604 to KPJ.

265

266 **Author contributions**

267 J.D., and K.P.J. conceived the study. J.D., A.D.S., and K.P.J. designed the study. A.D.S.

268 collected the data. J.D. and A.D.S. analysed the data. K.P.J. obtained financial support for the

269 project. J.D. wrote the manuscript and all authors contributed to editing the manuscript.

270

271 **Data and materials availability**

272 All data needed to evaluate the conclusions in the paper are present in the paper and/or the

273 Supplementary Materials. Additional data related to this paper may be requested from the

274 authors.

275

276 **Competing interests**

277 The authors declare that they have no competing interests.

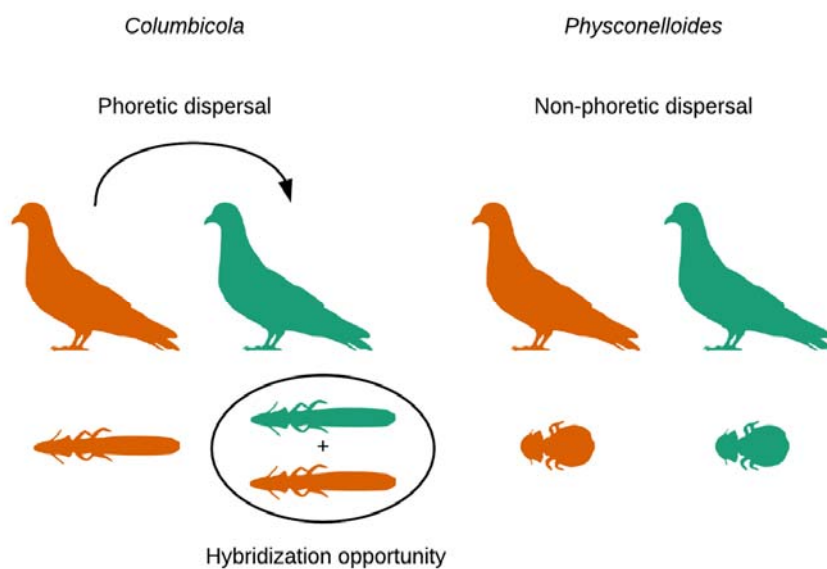
278

279 **Correspondence and requests for materials** should be addressed to J.D (jorged@illinois.edu)

280 or K.P.J. (kpjohnso@illinois.edu).

281

282 **Figure 1.** Diagram depicting the ecological replicate system and the hypothesis of this  
283 study. Wing lice (*Columbicola*) have higher dispersal abilities than body lice (*Physconelloides*),  
284 and thus higher odds of encountering individuals of a different louse species on the same host.  
285 Thus, wing lice are predicted to show higher levels of introgression compared to body lice.  
286

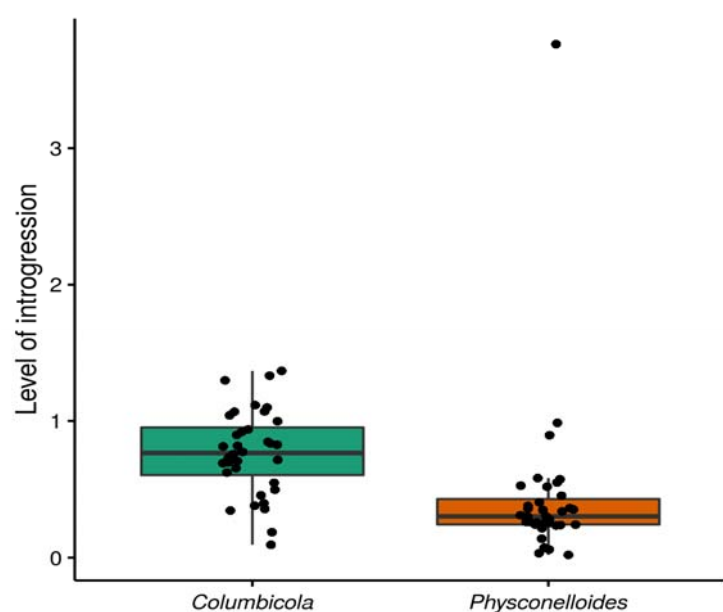


287

288

289 **Figure 2.** Boxplot showing the differences in levels of introgression between wing (green) and  
290 body (orange) lice. Level of introgression represents the sum of the mean coverage of  
291 reads mapped from all the species excluding the focal louse species, divided by the mean  
292 coverage of the focal louse species (see Methods). Black dots represent individual samples  
293 (horizontally jittered).

294



295

296



297 **Figure 3.** Optimal phylogenetic networks of feather lice genera. Orange branches depict  
298 reticulations. From left to right, *Columbicola* (seven reticulations) and *Physconelloides* (four  
299 reticulations) networks (See Methods).

