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1 **The evolution and diversity of intra-male sperm translocation in**
2 **Odonata: a unique behaviour in animals**

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4 Rivas-Torres, A.¹, Outomuro, D.²; Lorenzo-Carballa, M. O.¹ & Cordero-Rivera,
5 A.¹

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7 ¹*ECOEVO Lab, Universidade de Vigo, Escola de Enxeñaría Forestal, Campus*
8 *A Xunqueira, 36005 Pontevedra, Galiza, Spain*

9 ²*Department of Biological Sciences, University of Cincinnati, Rieveschl Hall,*
10 *45221, Cincinnati, Ohio, USA*

11

12 **Author for correspondence:** Anais Rivas-Torres; **email:** arivasto@gmail.com,
13 **address:** ECOEVO Lab, Universidade de Vigo, Escola de Enxeñaría Forestal,
14 Campus Universitario A Xunqueira, 36005 Pontevedra, Galiza, Spain;
15 **telephone:** +34657885598

16 **Abstract**

17 Behavioural diversity is a basic component of biodiversity, with implications in
18 ecological interactions at the intra- and interspecific levels. The reproductive
19 behaviour of Odonata (dragonflies and damselflies) is unique among insects
20 and conditioned by the anatomical separation between the male's reproductive
21 organs and the intromittent organ. Prior to mating, males must translocate
22 sperm from the genital pore in the 9th abdominal segment to the seminal vesicle
23 located ventrally in the 2nd abdominal segment. This behaviour, exclusive to
24 odonates, is known as intra-male sperm translocation (ST). Here, we review the
25 literature on ST, and use phylogenetic comparative analyses to investigate the
26 evolution of ST within the Odonata. Information on ST was compiled for 176
27 species, with the commonest variant being ST once per mating, after tandem
28 formation (66%). Other variants found were: ST involving precopulatory genital
29 touching (10%), ST by the male alone before tandem (16%) or after copulation
30 (5%), and repetition of ST during the same copulation (3%). The precopulatory
31 genital touching might have evolved to detect female receptivity. ST before
32 tandem formation might be favoured when mating opportunities are scarce and
33 copulations are brief. ST after mating might be favoured if males need to be
34 ready to copulate fast. Finally, repeated ST could have evolved through
35 postcopulatory sexual selection in males with limited sperm removal ability, as a
36 mean to improve their sperm competition. The most plausible scenario for the
37 evolution of ST is that the ancestors of the Odonata produced a spermatophore
38 and attached it to the body, leading towards the evolution of the secondary
39 genitalia in males. Our study emphasizes the role of behavioural diversity to
40 understand behavioural evolution.

41 **Significance statement**

42 Unique behaviours are exclusive of a few individuals, populations and/or
43 species. The intra-male sperm translocation (ST) of dragonflies and damselflies
44 is a unique behaviour in animals: before mating, males need to transfer sperm
45 from the primary to the secondary genitalia, which are anatomically separated.
46 Thus, the viability and quality of sperm (*i.e.* fertility) will depend on the timing of
47 ST relative to copulation. Our literature review found a variety of ST variants,
48 being ST in tandem and before copulation the ancestral strategy. We discuss
49 putative evolutionary routes for all the variants found and emphasize the
50 importance of retrieving detailed observations of such unique behaviours in the
51 field, which could help to better understand behavioural evolution in this insect
52 group. Behavioural diversity is rarely addressed by conservation strategies,
53 despite unique behaviours being at a higher risk of extinction.

54

55 **Keywords:** dragonflies, damselflies, reproductive behaviour, ethodiversity

56 **Introduction**

57 Behavioural diversity or ethodiversity (Cordero-Rivera 2017a) is a fundamental
58 level of phenotypic variability that can promote population stability in changing
59 environments (e.g. Dingemanse et al. 2004), increase adaptability at the
60 intraspecific level (Berger-Tal and Saltz 2016) and inform about evolutionary
61 processes. Ethodiversity can thus play an important role in species resilience
62 and consequently in how we can manage species for conservation strategies.
63 Unfortunately, while many conservation strategies address the extinction of
64 species and its possible cascading effects across trophic levels (Pérez-Méndez
65 et al. 2016), the disappearance of behaviours is rarely considered (Caro and
66 Sherman 2012). Behaviours that are exclusive of a limited number of
67 individuals, populations or species, are relevant from a conservation
68 perspective, because they are in high risk of extinction (Caro and Sherman
69 2012). For instance, sexual conflict can be an engine of speciation, because it
70 may trigger antagonistic coevolution between the sexes, potentially leading to
71 rapid divergent evolution of the characters related to reproduction (e.g. Cordero-
72 Rivera, 2017b). Therefore, the study of reproductive behaviours is relevant for
73 our understanding of diversification and speciation.

74 Insects from the order Odonata (dragonflies and damselflies) represent a clear
75 example of rare behavioural diversity, regarding their copulatory behaviour. In
76 most insects, copulation is performed with both partners oriented in opposite
77 directions (Figure 1A). However, the reproductive behaviour of odonates is
78 unique because during copulation male and female remain attached by two
79 points, forming the so-called “copulatory wheel” (Figure 1B). In this position, the
80 male anal appendages located at the tip of the abdomen grasp the female
81 thorax (or head), and the female genitalia located at the distal part of the
82 abdomen, contacts with the male secondary genitalia, positioned in the proximal
83 part of the abdomen. Such copulatory position occurs because the male
84 intromittent organ is situated in the second and third abdominal segments
85 (secondary genitalia), while the male primary genitalia are located in the ninth
86 abdominal segment. Therefore, before insemination, the male must translocate
87 sperm from the primary to the secondary genitalia, a behaviour called intra-male
88 sperm translocation (hereafter, ST) (Figure 1C-F). The ST behaviour is not

89 found in any other insect (Shuker and Simmons 2014; Córdoba-Aguilar et al.
90 2018), although indirect insemination is also found in octopuses and spiders.
91 The ST it is thus a clear example of diversity at the behavioural level with
92 important evolutionary implications, because the viability and quality of the
93 sperm, and hence fertility, will depend on the timing of ST relative to copulation
94 (Rivas-Torres et al. unpublished).
95 However, little is known about its evolution, its relation with the evolution of the
96 copulatory wheel, and particularly its diversity within the order. Our main aim
97 was therefore to explore the variation of ST behaviour from an evolutionary
98 perspective and discuss its possible connection with the copulatory wheel. We
99 performed a literature review on the diversity of ST across Odonata, and we
100 used phylogenetic comparative analyses to investigate the evolution of the ST
101 behaviour within this insect order. Our literature review revealed differences in
102 ST behaviour within and between species, and the comparative phylogenetic
103 analyses suggested ST in tandem and before copulation, as the ancestral
104 behaviour in Odonata. We discuss potential evolutionary routes for all the ST
105 behaviours found, which could help to better understand behavioural evolution
106 in this insect group, as well as certain aspects of the evolution of sexual
107 behaviours and divergence in other animals.

108

109 **Material and Methods**

110 *Literature review of ST behaviour*

111 Published data on ST in odonates were searched by querying Google scholar
112 (<http://scholar.google.com>) for “sperm translocation” and “odonat*” or “intra-
113 male sperm transfer” and “odonat*”. Searches were carried out in December
114 2016. Additional searches were completed in November 2018 looking for ST
115 behaviour in papers written in Spanish, French, German and Italian and
116 manually looking in old English references, where the behaviour could be
117 described in different terms (e.g. “filling of the sperm vesicle”). Japanese
118 references were also included thanks to the help of colleagues. We also
119 screened manually the papers published in *Martinia* (from 1991 to 2016) and in
120 the *Journal of the British Dragonfly Society* (from 1995 to 2016), which are two
121 non-digitized odonatological journals). We collated all available observations of

122 ST, including how this behaviour was performed, and its duration (with sample
123 size and standard error). We compiled information for a total of 176 species
124 (see Results). Full details of the literature reviewed, variables considered, and
125 species included in the analyses are given in Supplementary Materials 1 and 2.
126 For the purposes of our study, we have categorized ST in four characters: (i)
127 before or after copula, (ii) alone or in tandem, (iii) non-repeated or repeated,
128 and (iv) with or without precopulatory genital touching (see Table 1 and
129 Results). Since the description of the precopulatory genital touching might not
130 be stated explicitly in the revised papers, we established that when something
131 similar to this behaviour was described just before the ST, we considered it as
132 precopulatory genital touching.

133

134 *Phylogenetic tree reconstruction*

135 We aimed at understanding the evolution of ST using phylogenetic comparative
136 analyses (Harvey and Pagel 1991). Therefore, we first constructed a
137 phylogenetic tree including the odonate species for which data on ST were
138 available in the literature. We searched GenBank (Clark et al. 2016,
139 <https://www.ncbi.nlm.nih.gov/genbank/>) for sequences of the mitochondrial
140 genes COI and 16S, and the nuclear genes 18S and 28S. In total, we retrieved
141 sequence data for 129 species out of the 176 for which we had data on ST. We
142 also retrieved sequences of the same genes from 31 species without
143 information on ST, to increase the resolution of the tree, and for six species of
144 Ephemeroptera, to be used as outgroups in the analysis. The final dataset
145 included a total of 160 Odonata species (including 31 without data for ST),
146 which represented 14 families of Zygoptera and 11 families of Anisoptera (see
147 Supplementary Material Table S2).

148 All sequences were imported into Geneious version 9.1.7

149 (<http://www.geneious.com>, Kearse et al. 2012) for visual inspection before
150 alignment. Sequences were aligned using the ClustalW algorithm (Thompson et
151 al. 1994), as implemented in Geneious. We used BEAST version 2.4.8.0
152 (Bouckaert et al. 2014) to reconstruct the phylogenetic relationships among the
153 study species. The phylogenetic reconstruction was performed with the
154 nucleotide substitution models selected for each gene by the bModelTest

155 package version 1.0.4 (Bouckaert and Drummond 2017), and a strict clock
156 model and the Yule speciation model as priors. The analysis was run for 10
157 million generations and sampled every 1,000 generations. The output was
158 examined with Tracer version 1.6 (Rambaut et al. 2014) to assess convergence
159 of the Markov-chain Monte Carlo onto a stationary distribution through the
160 analysis of trace plots and effective sample sizes (ESS) of the model
161 parameters (ESS > 200 was considered acceptable). TreeAnnotator version
162 2.4.8, included in the BEAST package, was used to build a maximum clade
163 credibility tree after discarding 10% of sampled trees as burn-in. The consensus
164 tree was visualized and edited with FigTree version 1.4.2 (available at:
165 <http://tree.bio.ed.ac.uk/software/figtree/>). This tree was pruned to contain only
166 those species with available ST information using the R package ape (Paradis
167 et al. 2004), and the pruned tree was subsequently used for the phylogenetic
168 comparative analyses.

169

170 *Phylogenetic comparative analyses*

171 We estimated the ancestral states of our study discrete characters related to
172 sperm transfer using the function ace in the R package ape (Paradis et al.
173 2004). Our character states were ST performed (i) before vs. after copula; (ii) in
174 tandem vs. alone vs. both in tandem and alone; (iii) with repetition vs. with no
175 repetition vs. with and without repetition and (iv) with precopulatory genital
176 touching vs. without precopulatory genital touching vs. with and without
177 precopulatory genital touching (Robertson and Tennessen 1984). We note that
178 the combined states (*i.e.* both in tandem or alone) correspond to intra- or
179 interpopulation variation of the ST behaviour. We used maximum likelihood
180 estimation with equal rates of transition (Pagel 1994) and the likelihood of the
181 ancestral states was computed using a joint estimation procedure (see Pupko et
182 al. 2000). Finally, we also investigated possible correlated evolution between
183 pairs of characters. For this analyses, we first recoded the study characters to
184 binary. We coded the combined states to the least common variant of ST for
185 each character. Our rationale behind this decision is that we wanted to give
186 more weight to the least common behaviours, which are already under-
187 represented in our dataset. Even though species with more than one type of ST

188 have not stably evolved only one ST behaviour, they could offer hints on the
189 evolution of ST across odonates. We used Pagel's (1994) function to detect
190 correlated evolution as implemented in the R package phytools (Revell 2012),
191 with the function fitDiscrete from the package geiger (Harmon et al. 2008).

192

193 **Results**

194 *Variability of ST behaviour*

195 We found a total of 123 papers that provided information on ST from 176
196 species of odonates, belonging to 22 families (Table 1, Supplementary
197 Materials 1 and 2). The ST is a behaviour that usually lasts for some seconds
198 but shows high variability (mean \pm SE: 16.7 \pm 2.7 sec, range: 0.2 to 150 sec, N
199 = 82 species) (Table 1).

200 At least five different variants of ST were described across species: (i) male
201 alone, before the formation of precopulatory tandem; (ii) in precopulatory
202 tandem, without genital touching, only once per copula; (iii) in precopulatory
203 tandem after genital touching, only once per copula; (iv) in tandem, repeated
204 during copulation; and (v) male alone, after copula (see Table 1). However, in
205 some species [e.g. *Libellula quadrimaculata* (L., 1758) or *Erythemis*
206 *simplicicollis* (Say, 1839)] we found a combination of the ST variants due to
207 intra- or interpopulation variability (Figure 2).

208 Overall, the majority of species (66%) perform ST in precopulatory tandem
209 (Table 1), hence before copulation and only once per copula. Anisoptera males
210 perform ST alone, before tandem formation in a higher proportion (31%)
211 compared with Zygoptera (9%). The ST in tandem and repeated was found for
212 4% of species of Zygoptera, but no in Anisoptera (Table 1). Finally, ST alone,
213 after copula was found for 6% of the species of Zygoptera and 3% of Anisoptera
214 (Table 1).

215 The families Coenagrionidae and Libellulidae, are the most speciose and also
216 display more variants of ST behaviour, including all five in the first family. The
217 majority of coenagrionids studied performed ST in precopulatory tandem, and
218 only once per copula (58%; Table 1). The second most common variant was the
219 ST in tandem, but only after precopulatory genital touch (32%; Table 1). In
220 libellulids, ST in tandem, only once per copula, was also the commonest variant

221 (61%; Table 1), and the second commonest ST was male alone, but in this
222 case, before tandem formation (33%; Table 1). ST by the male alone, after
223 copulation is a rare behaviour, but is the dominant behaviour in the Euphaeidae
224 (57%) and Polythoridae (67%) and it is also performed by the only living
225 representative of Pseudolestidae.

226

227 *Evolution of ST behaviour*

228 DNA sequences were available for 129 out of the 176 species for which
229 information on ST behaviour was available in the literature (Supplementary
230 Material Table S2). The consensus phylogenetic tree was congruent with the
231 currently accepted and relatively well-established phylogeny of the order (*e.g.*
232 Lorenzo-Carballa and Cordero-Rivera, 2014, but see Dijkstra et al. 2013), and
233 the support for the main clades was high in most cases (Supplementary
234 Material Figure S1).

235 In our dataset, the majority of species perform ST before copula (Figure 2). Six
236 species perform ST after copula (four Zygoptera and two Anisoptera).
237 According to the ancestral reconstruction for this trait, the variant of ST after
238 copula has evolved independently in all cases except for the group
239 Euphaea/Anisophaea (Zygoptera). Regarding ST in tandem and/or alone, most
240 species perform it in tandem, while 22 species do it alone (seven Zygoptera and
241 15 Anisoptera), and nine species use both strategies (three Zygoptera and six
242 Anisoptera). These variants have independently evolved in all cases except for
243 the group *Euphaea/Anisophaea* (Zygoptera), and the clade
244 *Nannothemis/Pachydiplax/Erythemis* and for the species of *Leucorrhinia*
245 (Anisoptera). In the case of ST performed repeatedly or not, we found that the
246 majority of species perform it just once. Only within Zygoptera, we found three
247 species that repeat sperm transfer and two that perform both variants
248 (corresponding to five independent origins). Regarding ST performed with or
249 without genital touching, only five species have ST with genital touching and
250 three perform both variants (all of them within Zygoptera). These cases
251 correspond to independent origins except for the group of *Ischnura ramburii*, *I.*
252 *graellsii* and *I. elegans*. In summary, the ancestral state of ST would be before
253 copula, in precopulatory tandem, non-repeated and without genital touching.

254 Finally, we did not find any significant correlated evolution between the different
255 variants of ST ($P > 0.05$).

256

257 **Discussion**

258 Our review of the literature indicates that there are at least five variants of ST
259 behaviour among Odonata. Our work also highlights the lack of basic
260 information on the ST behaviour for the vast majority of species within this
261 insect group: the total number of odonate species is estimated around 6000
262 (Lorenzo-Carballa and Cordero-Rivera, 2014), however we only found data on
263 ST behaviour for 176 species.

264 **Variation of ST behaviours**

265 In most of the odonate species studied, males perform ST after having grasped
266 the female in precopulatory tandem and once per copula (variant ii). This variant
267 of ST is the ancestral state, according to our comparative phylogenetic
268 analyses. Variant (i), the completion of ST by the male alone, before finding a
269 female (Table 1), could be advantageous in species where females are rarely
270 encountered and copulations are brief. Males that performed ST alone would be
271 ready to copulate as soon as they grasp a female in tandem.

272 Variant (iii), involves performing ST only after precopulatory genital touching
273 (Table 1), a behaviour that presumably signals female receptivity to the male
274 (Robertson and Tennessen, 1984). This behaviour might derive from the basal
275 behaviour (*i.e.* variant ii), if males commonly encounter unreceptive females.

276 High densities of unreceptive females would be predicted in populations/species
277 whose females remain near the reproductive site in the maturation phase, like
278 many *Ischnura* (Cordero et al., 1998). When females give no refusal signs,
279 males can remain in tandem for very long periods, even for a full day in the
280 laboratory (Cordero et al. 1992). Genital touching would therefore be adaptive
281 for males, avoiding wasting sperm with unreceptive females, but also for
282 females, because they would be released faster if signalling their
283 unreceptiveness. In odonates, it has been assumed that females cannot be
284 forced to copulate (e.g. Fincke 1997), although forced matings are possible in
285 populations with high male densities and females ovipositing unguarded
286 (Cordero-Rivera and Andrés 2002).

287 Only two species, *Coenagrion scitulum* (Rambur, 1842) and *Megaloprepus*
288 *caerulatus* (Drury, 1782), are known to routinely perform variant (iv), i.e.
289 repeated ST in one copulation (Table 1, Figure 1D). This behaviour has been
290 also recorded occasionally in three other zygopteran species: *Lestes barbarus*
291 (Fabricius, 1798) (Lestidae), *Ischnura aurora* (Brauer, 1865) (Coenagrionidae)
292 and *Perissolestes remotus* (Williamson & Williamson, 1924) (Perilestidae) (see
293 Supplementary Material 1). In the case of *C. scitulum*, the repetition of ST
294 during the copulation has been interpreted as a mechanism evolved in the
295 context of sperm competition (Córdoba-Aguilar and Cordero-Rivera 2008). In
296 this species, males are apparently unable to remove a significant portion of
297 sperm from rivals using their genital ligula (Cordero et al. 1995). Therefore, by
298 repeating ST and insemination, they might over-compete rival sperm. However,
299 other species of odonates [e.g. *Hypolestes trinitatis* (Gundlach, 1888)] are also
300 known to have limited ability to remove sperm, but ST is not repeated (Torres-
301 Cambas and Cordero-Rivera 2011), and hence the link between both
302 phenomena is not straightforward.

303 The most intriguing variant for ST is variant (v), i.e. the translocation of sperm
304 by the male alone, after copulation (Figure 1F, Table 1). This behaviour was
305 first reported for the coenagrionid *Mortonagrion Hirosei* Asahina, 1972, from
306 Japan (Naraoka 2014), and then observed in nine additional species, including
307 four Euphaeidae, and *Pseudolestes mirabilis* Kirby, 1900 (Cordero-Rivera and
308 Zhang 2018). Translocating sperm after copulation could be explained if these
309 species have evolved physiological mechanisms to maintain sperm alive for
310 long periods of time (until next mating). However, at least for the first copula,
311 males have to fill their sperm vesicle before copulation. Males could routinely
312 perform ST each morning to be prepared for the next mating, but this has never
313 been observed in *P. mirabilis*, the only species studied in detail (Cordero-Rivera
314 and Zhang 2018).

315 In some species, we found several of the ST variants due to intra- or
316 interpopulation variability. For example, males of *Hetaerina americana*
317 Fabricius, 1798 were reported performing ST alone before copula in one
318 population, in tandem without precopulatory touch in another population and in
319 tandem with precopulatory touch at a third locality (Supplementary material

320 Table 1). Two ST variants were also reported for different populations of *I.*
321 *aurora* and *Libellula quadrimaculata* Linnaeus, 1758, and between males of a
322 single population of *Aeshna cyanea* Muller, 1764 (Supplementary material
323 Table 1). Intraspecific variability seems rare, and unlikely to be detected in
324 short-term studies. For instance, only 5% of the 137 ST observed in two
325 *Enallagma* species occurred before tandem formation (Logan, 1971; cited by
326 Corbet, 1999). The reasons behind this intraspecific diversity of ST are
327 unknown, but these species are excellent candidates for further investigation of
328 the diversification of ST strategies within odonates.

329 **Why has ST evolved?**

330 The evolution of ST behaviour is likely related to the atypical copulation position
331 in the Odonata. One plausible scenario for the evolution of ST behaviour is that
332 ancestors of modern odonates produced a spermatophore, and deposited it on
333 the substrate, a behaviour currently observed in arachnids, myriapods and
334 wingless hexapods (Proctor 1998). The thick cerci of *Namurotypus* Brauckmann
335 and Zessin, 1989 males (ancestor of the carboniferous Odonata) could have
336 been used to firmly grasp the female behind her compound eyes. The male
337 could then have directed the female over the spermatophore (Bechly et al.
338 2001), in a way similar to the 'drag off' behaviour observed in whipspiders
339 (Amblypygi) (Weygoldt 1969). Attaching the spermatophore to the male body is
340 clearly more efficient, and could be the selective pressure required for the
341 evolution of secondary genitalia in odonates (reviewed in Cordero-Rivera and
342 Córdoba-Aguilar 2010), and hence, the ST behaviour.

343 An alternative, yet more speculative hypothesis for the evolution of ST could be
344 related to sexual cannibalism, since females of several zygopterans are known
345 to sometimes attack and eat mature conspecific males (Cordero 1992). We are
346 aware of only one case of sexual cannibalism described during copulation:
347 Robertson (1985) observed a female of *Ischnura ramburi* (Selys, 1850) that was
348 repeatedly chased and grasped by a male until she finally attacked him and ate
349 out his thorax, but the male succeeded initiating copulation before dying in
350 copula. Other predatory animals, like spiders or octopuses, have a specialised
351 appendix used as an intromittent organ to introduce the spermatophore in the
352 female reproductive organs. These two groups also show sexual cannibalism

353 (Ibáñez and Keyl 2010; Li et al. 2012). Therefore, it could be hypothesised that
354 the tandem and wheel position of odonates during copulation, along with the
355 intra-male ST, might allow males to avoid sexual cannibalism (Chapman et al.
356 2003; Schneider 2014). From this idea, we propose the hypothesis that when
357 the risk of sexual cannibalism is high, selection favours the evolution of
358 secondary mechanisms to inseminate females safely for the males. This could
359 be tested with a review of the insemination behaviours across different animal
360 taxa and within a phylogenetic context.

361 **Conclusions**

362 Some of the ST variants seem to have evolved several times, but this behaviour
363 needs further investigation in a larger number of species. No other insect group
364 shows a behaviour equivalent to the ST of odonates (although some similarities
365 can be found with the pedipalps of spiders and the hectocotylus of octopuses),
366 and consequently we lack comparative evidence to understand the evolution of
367 this enigmatic behaviour. ST is sometimes performed very fast and therefore
368 careful video recording of this behaviour, particularly in Anisoptera, is needed to
369 avoid confusing movements to clean the abdomen with true ST. We currently
370 lack information for a number of families of both Anisoptera (e.g.
371 Austropetaliidae, Neopetallidae and Macromiidae) and Zygoptera (e.g.
372 Chlorocypgidae, Amphipterygidae and Lestoideidae), and most of the species
373 with information on ST are from temperate zones and fewer from tropical areas.
374 This bias can be explained by the scarcity of field studies in tropical regions,
375 and also due to the low sexual activity observed in tropical species (e.g.
376 Sanmartín-Villar and Cordero-Rivera, 2016).

377 Our study emphasizes also the relevance (and scarcity) of detailed natural
378 history observations for most species. We expect that this review will encourage
379 the scientific community towards more research in diversity of reproductive
380 behaviours, with a special focus on tropical species. This applies not only to
381 odonates, but to other animal taxa. This information, combined with modern
382 molecular techniques and phylogenetic hypotheses, is fundamental to
383 understand relevant questions about behavioural evolution as well as
384 behavioural diversity (ethodiversity). This is a necessary step to increase

385 awareness on the importance of conserving not only species, but also
386 behaviours (Cordero-Rivera, 2017a).

387

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397

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Figure captions

Fig. 1: Insects usually mate with **both** sexes facing opposite directions, like in butterflies (**A**: *Hipparchia* sp. Fabricius, 1807; Satyridae). However, in odonates (**B**: *Orthetrum brunneum* (Fonscolombe, 1837), Libellulidae), the sexes are orientated to the same direction, allowing flying in copula. The intra-male sperm translocation can take place after the male has grasped the female in tandem, like in (**C**) *Neurobasis chinensis* (Linnaeus, 1758) (Calopterygidae), with or without precopulatory genital touching. In some species, this behaviour is repeated up to seven times in the same copulation, like in (**D**) *Coenagrion scitulum* (Rambur, 1842) (Coenagrionidae). Finally, **in some species males translocate sperm alone before copulation, like in (E) *Diphlebia lestoides* (Selys, 1853),** or after copulation, very close to the female as in (**F**) *Euphaea masoni* Selys, 1879 (Euphaeidae). Pictures: A. Cordero-Rivera, except (E), courtesy of Reiner Richter.

Fig. 2: The **variants** of intra-male sperm translocation behaviour, mapped on a phylogeny of the studied Odonata. The ancestral behaviour of sperm translocation was estimated to be before copula, in precopulatory tandem, non-repeated and without genital touching. **Family names have been added to the main branches of the tree.**

Figure 1

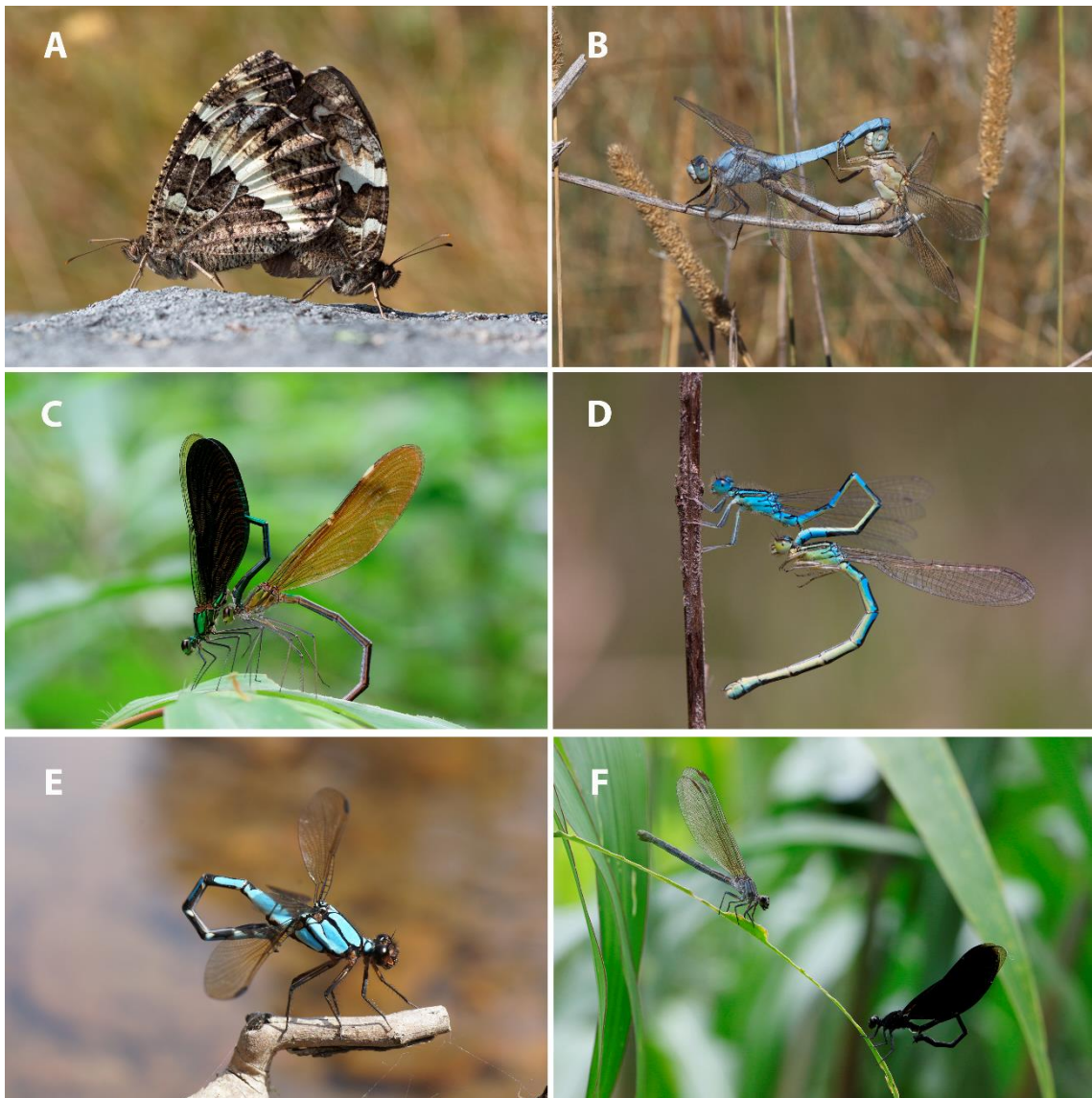
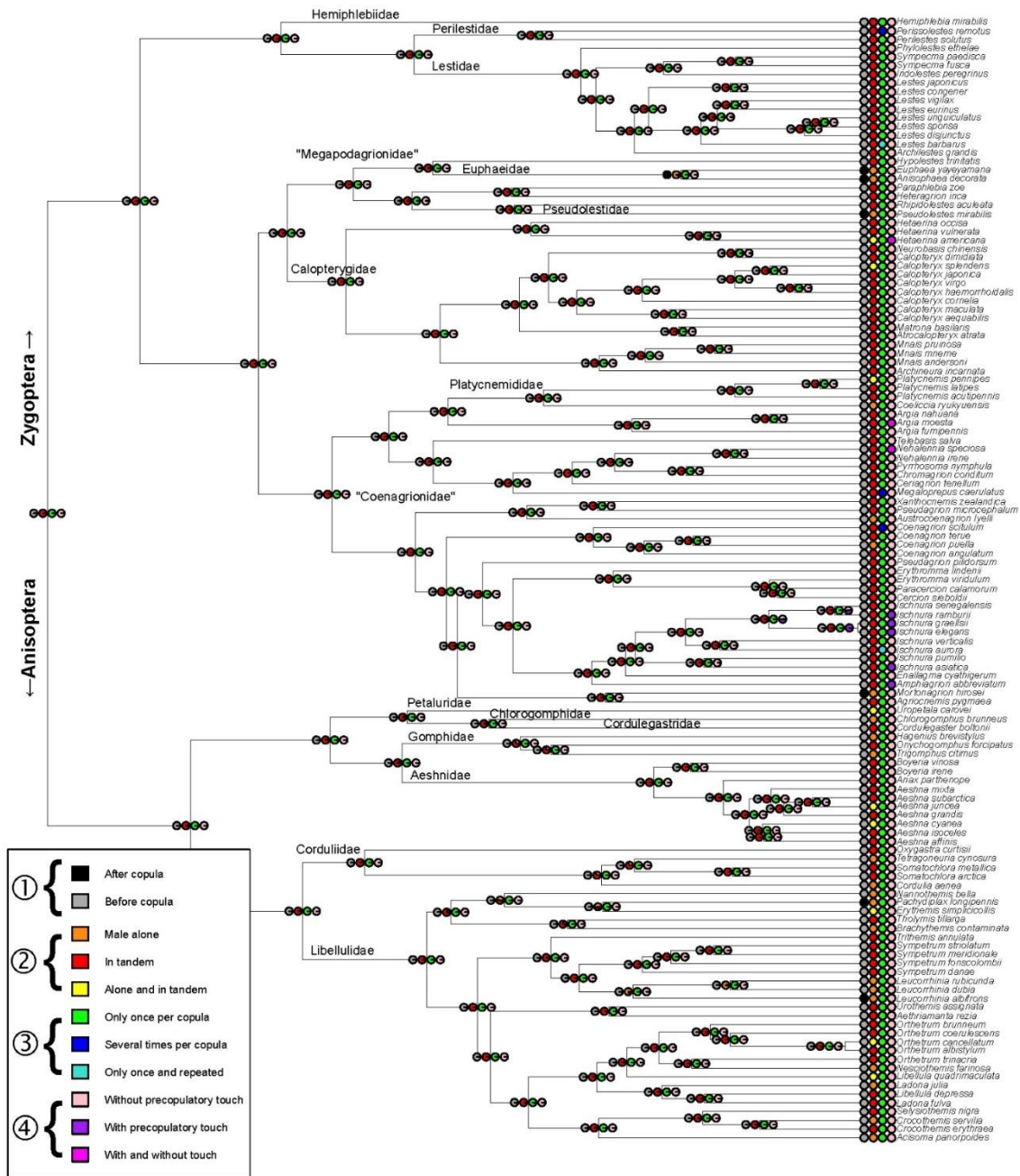


Figure 2



The evolution and diversity of intra-male sperm translocation in Odonata: a unique behaviour in animals

Rivas-Torres, A.¹, Outomuro, D.²; Lorenzo-Carballa, M. O.¹ & Cordero-Rivera, A.¹

¹ECOEVO Lab, Universidade de Vigo, Escola de Enxeñaría Forestal, Campus A Xunqueira, 36005 Pontevedra, Galiza, Spain

² Department of Biological Sciences, University of Cincinnati, Rieveschl Hall, 45221, Cincinnati, Ohio, USA

Author for correspondence: Anais Rivas-Torres, **email:** arivasto@gmail.com, **address:** ECOEVO Lab, Universidade de Vigo, Escola de Enxeñaría Forestal, Campus A Xunqueira, 36005 Pontevedra, Galiza, Spain, **telephone:** +34657885598

Gomphidae	2	66.7	1	33.3	0	0	0	0	0	0	3	5.0 (1)
Libellulidae	12	33.3	22	61.1	0	0	0	0	2	5.6	36	5.3±4.6 (13)
Petaluridae	1	50.0	1	50.0	0	0	0	0	0	0	2	
Anisozygoptera	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	1	
Epiophlebiidae	0	0	1	100	0	0	0	0	0	0	1	
TOTAL	31	16.0	128	66.0	20	10.3	5	2.6	10	5.2	194	16.7±2.7 (82)