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Supplementary data to:

The evolutionary history of colour polymorphism in *Ischnura* damselflies (Odonata: Coenagrionidae)

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Methodological details. DNA extraction and sequencing

Previous phylogenetic studies included 15 Nearctic and Neotropical taxa (Chippindale et al. 1999) and 24 old-world species (mainly Palaearctic taxa; Dumont 2013). We included 44 taxa in our study, and used two mitochondrial genes, cytochrome oxidase II (COII) and cytochrome b (CYTB), and a part of two nuclear genes, the small sub-unit 18S nrDNA (ITS1) and 5.8S (ITS2), and the large sub-unit rDNA 28 nrDNA (18S-ITS). For species

lacking published sequences (*Ischnura* sp. "a", *I. chingaza*, *I. cruzi*, *I. cyane*, *I. foylei*, and *I. indivisa*), DNA was extracted from the thorax using a standard phenol/chloroform-isoamylalcohol extraction protocol (Sambrook et al. 1989). Table S1 shows the corresponding sequence accession numbers. DNA samples were amplified by PCR using universal primers: TL2-J-3037 and C2-N-3494 and C2-J-3400 and TK-N-3785 (n = 673 bp) for COII (Simon et al. 1994); CB-J-10933 and TS1-N-11683 (n = 457 bp) for CYTB (Simon et al. 1994); and: ITS-1 and reverse 28H (n = 751 bp) for 18S-ITS (Samraoui et al. 2002). DNA amplifications were done following the protocol described in Sánchez-Guillén et al. (2014). Bidirectional sequencing was conducted, and the sequencing was completed on an ABI 3 100 Genetic Analyzer. Because we have sequenced DNA from five dried specimens with partially degraded DNA, the amplification of the tree genes was not possible in the six species (*cf.* Table S1). Forward and reverse sequences were edited in GeneStudio v.2.2.0.0. Variable positions were revised by eye, and, based on the chromatogram, only high-quality sequences were considered. Sequenced data from old or dried specimens may be incomplete due to degraded DNA. Therefore, we only processed high-quality sequences for each gene and taxon and then selected the longest sequences of these to maximize data coverage (GenBank accession numbers, Table S1).

References

Chippindale P.T., Dave V.K., Whitmore D.H. & Robinson J.V. 1999. Phylogenetic relationships of North American damselflies of the genus *Ischnura* (Odonata: Zygoptera: Coenagrionidae) based on sequences of three mitochondrial genes. *Molecular Phylogenetics and Evolution* 11: 110-121

Dumont H.J. 2013. Phylogeny of the genus *Ischnura* with emphasis on the Old World taxa (Zygoptera: Coenagrionidae). *Odonatologica* 42: 301-308

Sambrook J., Fritsch E.F. & Maniatis T. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York

Samraoui B., Weekers P.H.H. & Dumont H.J. 2002. The *Enallagma* of the Western and Central Palaearctic (Zygoptera: Coenagrionidae). *Odonatologica* 31: 371-381

Sánchez-Guillén R.A., Córdoba-Aguilar A., Cordero-Rivera A. & Wellenreuther M. 2014. Genetic divergence predicts reproductive isolation in damselflies. *Journal of evolutionary Biology* 27: 76-87

Simon C., Frati F., Beckenbach A., Cresp B., Liu H. & Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America* 87: 651-701

Table S1. Sequence references and GenBank accession numbers. Morgan (2002) – data from: Morgan J.L. 2002. Worldwide phylogeny of the damselfly genus Ischnura based on mitochondrial cytochrome oxidase II and cytochrome B sequence data. Master thesis, The University of Texas at Arlington; na – data were not available.

Species	References		
	Gene COII	Gene CYTB	Gene ITS
Ischnura sp. "a"	na	MG561437	na
Ischnura abyssinica	na	na	FN356098.1
Ischnura aralensis	na	na	FN356099
Ischnura asiatica	KC430145	KC430194	AB706602
Ischnura aurora	Morgan (2002)	Morgan (2002)	AB706608
Ischnura barberi	AF067663	AF067683	na
Ischnura capreolus	AF067682	AF067684	na
Ischnura cervula	AF067665	AF067685	FN356101
Ischnura chingaza	na	MG561433	MG561438
Ischnura cruzi	na	MG561435	MG561439
Ischnura cyane	na	MG561434	MG561440
Ischnura damula	AF067666	AF067686	na
Ischnura demorsa	AF067667	AF067687	na
Ischnura denticollis	AF067668	AF067688	FN356102
Ischnura elegans	HQ834805	HQ834800	KC430216
Ischnura e. ebneri	JQ911513	KC430182	JQ911510
Ischnura erratica	AF067671	AF067691	na
Ischnura evansi	Morgan (2002)	Morgan (2002)	na
Ischnura ezoin	na	na	AB706574
Ischnura fluviatilis	Morgan (2002)	Morgan (2002)	na
Ischnura foylei	MG561432	na	na
Ischnura forcipata	na	na	FN356104.1
Ischnura fountaineae	KC430124	KC430174	KC430212
Ischnura gemina	AF067672	AF067692	na
Ischnura genei	KC430116	KC430152	KC430199
Ischnura graellsii	HQ834807	KC430158	KC430203
Ischnura hastata	AF067673	AF067693	na
Ischnura heterosticta	Morgan (2002)	Morgan (2002)	AB706612
Ischnura indivisa	na	MG561436	na

Species	References		
	Gene COII	Gene CYTB	Gene ITS
Ischnura intermedia	Morgan (2002)	KY127445	na
Ischnura kellicotti	AF067675	AF067695	na
Ischnura nursei	na	na	MH447413
Ischnura perparva	AF067676	AF067696	FN356106
Ischnura posita atezca	AF067677	AF067697	na
Ischnura p. posita	na	AF067698	na
Ischnura prognata	AF067679	AF067699	na
Ischnura pumilio	KC430197	KC430198	KC430231
Ischnura ramburii	AF067680	AF067700	na
Ischnura rubilio	na	na	MH447434
Ischnura rufostigma	na	na	AB706613
Ischnura saharensis	KC430123	KC430168	KC430211
Ischnura senegalensis	KC430142	KC430189	KC430219
Ischnura ultima	Morgan (2002)	Morgan (2002)	na
Ischnura verticalis	AF067682	AF067702	na
Enallagma basidens	na	AF067689.1|	na
Enallagma civile	na	AF067690.1|	na

Table S2. Species distribution and biogeographic realms
Information was taken from the following multiple databases providing open access to biodiversity data:
GBIF – Global Biodiversity Information Facility.
Online on the Internet, URL (30-x-2020): http://www.gbif.org/species/1423281
Dragonfiles 0.9.
Online on the Internet, URL (30-x-2020): http://www.medusa.jcu.edu.au
COL – Catalogue of Life.
Online on the Internet, URL (30-x-2020): www.catalogueoflife.org
EOL – Encyclopedia of Life.
Online on the Internet, URL (30-x-2020): https://eol.org/

Species	Distribution range	Biogeographic realms
Ischnura sp. „a“	Colombia	Neotropical
Ischnura abyssinica Martin, 1908	Ethiopia	Afrotropical
Ischnura acuticauda Lieftinck, 1959	New Guinea	Australasian
Ischnura albistigma Fraser, 1927	Samoa	Australasian
Ischnura aralensis Haritonov, 1979	Kazakhstan, Russia, Uzbekistan	Palaearctic
Ischnura ariel Lieftinck, 1949	New Guinea	Australasian
Ischnura armeniacum Lieftinck, 1949	New Guinea	Australasian
Ischnura asiatica (Brauer, 1865)	East Asia	Palaearctic
Ischnura aurora (Brauer, 1865)	Asia, Australia, Pacific	Australasian-Indomalayan
Ischnura barberi Currie, 1903	SW North America, Mexico	Nearctic
Ischnura buxtoni Fraser, 1927	Samoa	Australasian
*Ischnura capreo*lus (Hagen, 1861)	Central-South America	Neotropical
Ischnura cardinalis Kimmins, 1929	Society Islands (Polynesia)	Australasian
Ischnura cervula Selys, 1876	W North America	Nearctic
Ischnura chingaza Realpe, 2010	Colombia	Neotropical
Ischnura chromostigma Fraser, 1927	Samoa	Australasian
Ischnura cruzi De Marmels, 1987	Colombia	Neotropical
Ischnura cyane Realpe, 2010	Colombia	Neotropical
Ischnura damula Calvert, 1902	W North America	Nearctic
Ischnura demorsa (Hagen, 1861)	W North America	Nearctic
Ischnura denticollis (Burmeister, 1839)	W North America, Mexico	Nearctic
Ischnura dorothea Fraser, 1924	China	Palaearctic

Species	Distribution range	Biogeographic realms
Ischnura elegans (Vander Linden, 1820)	Eurasia	Palaearctic
Ischnura erratica Calvert, 1895	W North America	Nearctic
Ischnura evansi Morton, 1919	Iran, Iraq	Palaearctic
Ischnura ezoin (Asahina, 1952)	Ogasawara Islands (Japan)	Palaearctic
Ischnura filosa Schmidt, 1951	Madagascar	Afrotropical
Ischnura fluviatilis Selys, 1876	South America	Neotropical
Ischnura forcipata Morton, 1907	Asia	Palaearctic
Ischnura fountaineae Morton, 1905	North Africa, Pantelleria	Palaearctic
Ischnura foylei Kosterin, 2015	Sumatra	Indomalayan
Ischnura gemina (Kennedy, 1917)	California	Nearctic
Ischnura genei (Rambur, 1842)	Tyrrhenian Islands, Sicily, Maltese Islands	Palaearctic
Ischnura graellsii (Rambur, 1842)	Iberia, North Africa	Palaearctic
Ischnura haemastigma Fraser, 1927	Samoa	Australasian
Ischnura halecarpenteri Mundford, 1942	Marquesas Islands (Polynesia)	Australasian
Ischnura hastata (Say, 1839)	America, Azores	Neotropical-Nearctic
Ischnura heterosticta (Burmeister, 1839)	Australia	Australasian
Ischnura inarmata Calvert, 1898	Kashmir	Palaearctic
Ischnura indivisa (Ris, 1918)	Costa Rica	Neotropical
Ischnura intermedia Dumont, 1974	Turkey	Palaearctic
Ischnura isoetes Lieftinck, 1949	New Guinea	Australasian
Ischnura jeanyvesmeyeri Englund & Polhemus, 2010	Polynesia	Australasian
Ischnura karafutonis Matsumura, 1931	Japan	Palaearctic
Ischnura kellicotti Williamson, 1898	W North America	Nearctic
Ischnura lobata Needham, 1930	China	Palaearctic
Ischnura lorentzi Ris, 1913	New Guinea	Australasian
Ischnura luta Polhemus, Asquith & Miller, 2000	Rota (Polynesia)	Australasian
Ischnura mahechai Machado, 2012	Colombia	Neotropical
Ischnura nursei Morton, 1907	Asia	Palaearctic
Ischnura ordosi Bartenev, 1912	Asia	Palaearctic
Ischnura oreadum Lieftinck, 1949	New Guinea	Australasian

Species	Distribution range	Biogeographic realms
Ischnura pamelae Vick & Davies, 1988	New Caledonia	Australasian
Ischnura patricia Fraser, 1924	India	Indomalayan
Ischnura perparva McLachlan in Selys, 1876	W North America	Nearctic
Ischnura posita (Hagen, 1861)	E North America; introduced to Hawaii	Nearctic
Ischnura prognata (Hagen, 1861)	E North America	Nearctic
Ischnura pruinescens (Tillyard, 1906)	N Australia	Australasian
Ischnura pumilio (Charpentier, 1825)	Europe, Azores, Madeira	Palaearctic
Ischnura ramburii (Selys in Sagra, 1857)	North and Central America; introduced to Hawaii	Neotropical-Nearctic
Ischnura rhodosoma Lieftinck, 1959	New Guinea	Australasian
Ischnura rubella Navás, 1934	Asia	Palaearctic
Ischnura rubilio Selys, 1876	Asia (India, Bhutan)	Indomalayan
Ischnura rufostigma Selys, 1876	Asia	Palaearctic
Ischnura rufovittata (Blanchard, 1843)	South America	Neotropical
Ischnura rurutana Englund & Polhemus, 2010	Austral islands (Polynesia)	Australasian
Ischnura saharensis Aguesse, 1958	North Africa, Canary Islands	Palaearctic
Ischnura sanguinostigma Fraser, 1953	Samoa	Australasian
Ischnura senegalensis (Rambur, 1842)	Africa, Asia, Canary Islands	Afrotropical-Palaearctic
Ischnura sobrina Schmidt, 1942	Peru	Neotropical
Ischnura solitaria Bota-Sierra, Velásquez-Vélez & Realpe, 2019	Colombia	Neotropical
Ischnura spinicauda Brauer, 1865	Polynesia	Australasian
Ischnura stueberi Lieftinck, 1932	New Guinea	Australasian
Ischnura taitensis Selys, 1876	Tahiti	Australasian
Ischnura thelmae Lieftinck, 1966	Rapa island (Polynesia)	Australasian
Ischnura ultima Ris, 1908	Argentina	Neotropical
Ischnura verticalis (Say, 1839)	E North America, Mexico	Nearctic
Ischnura vinsoni Fraser, 1949	Mauritius	Afrotropical
Ischnura xanthocyane Lieftinck, 1949	New Guinea	Australasian

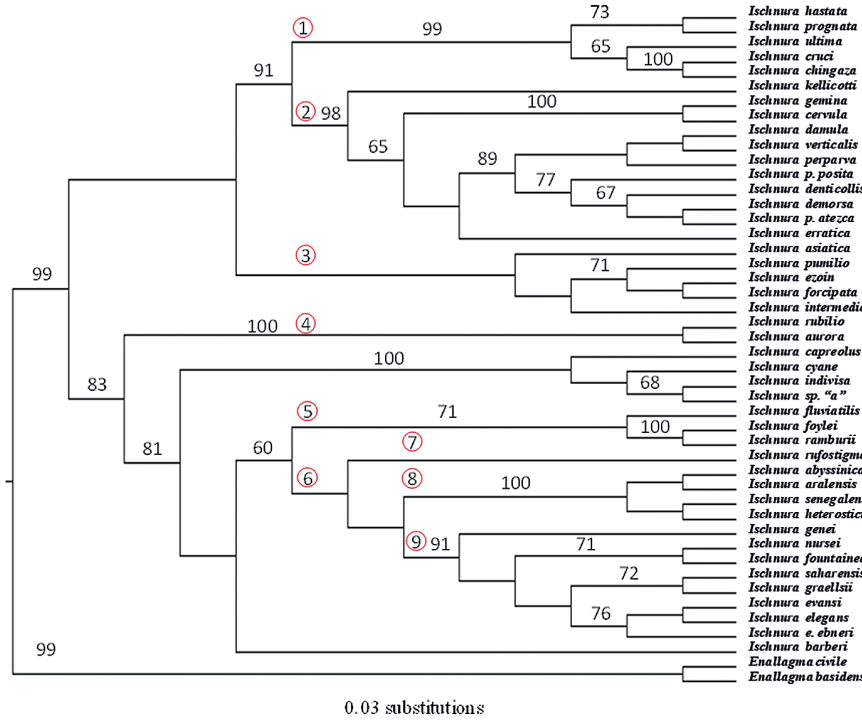

Figure S1. *Ischnura* phylogeny, including 10 novel taxa, by using maximum likelihood inference**.**

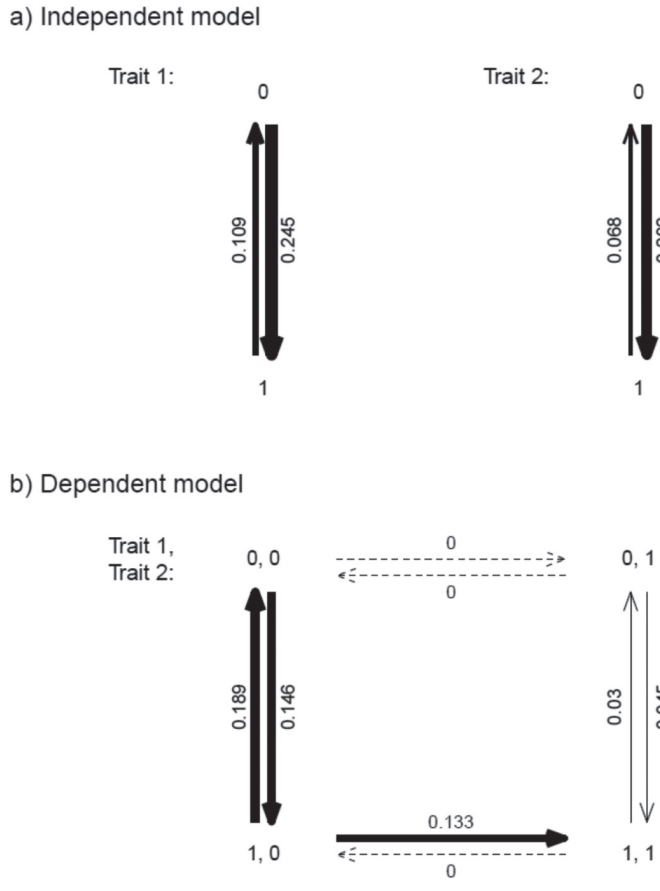

Figure S2. Correlation between female-limited colour polymorphism and mating system. Graphical representation of the transition rates between traits (females colour polymorphism and mating system) for the independent and the dependent models. Arrow widths are scaled to their average transition rates in that state.

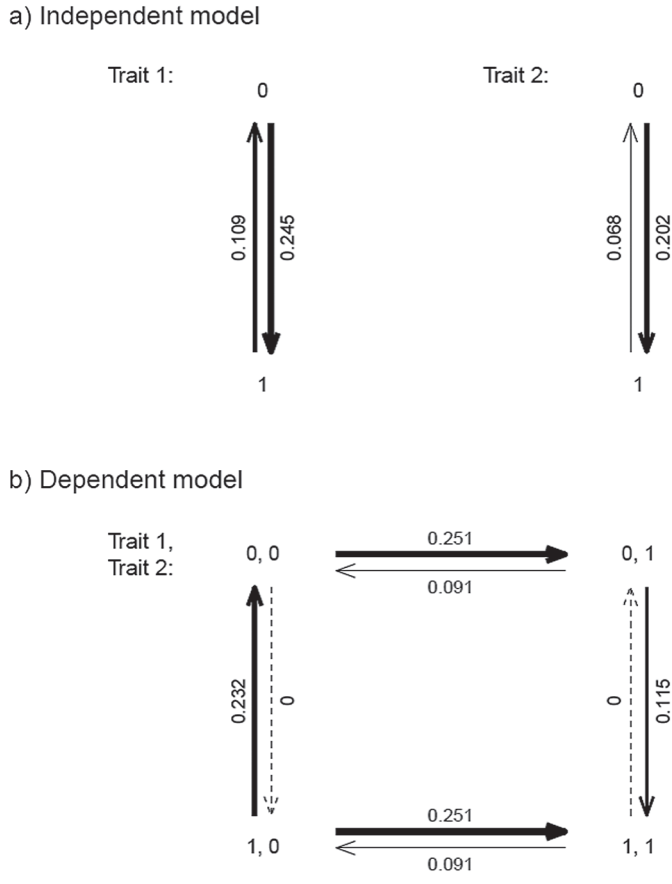

Figure S3. Graphical representation of the transition rates between traits (female colour polymorphism and mating system) for the independent and the dependent models. Arrow widths are scaled to their average transition rates in that state (female colour morphs depend on mating system).

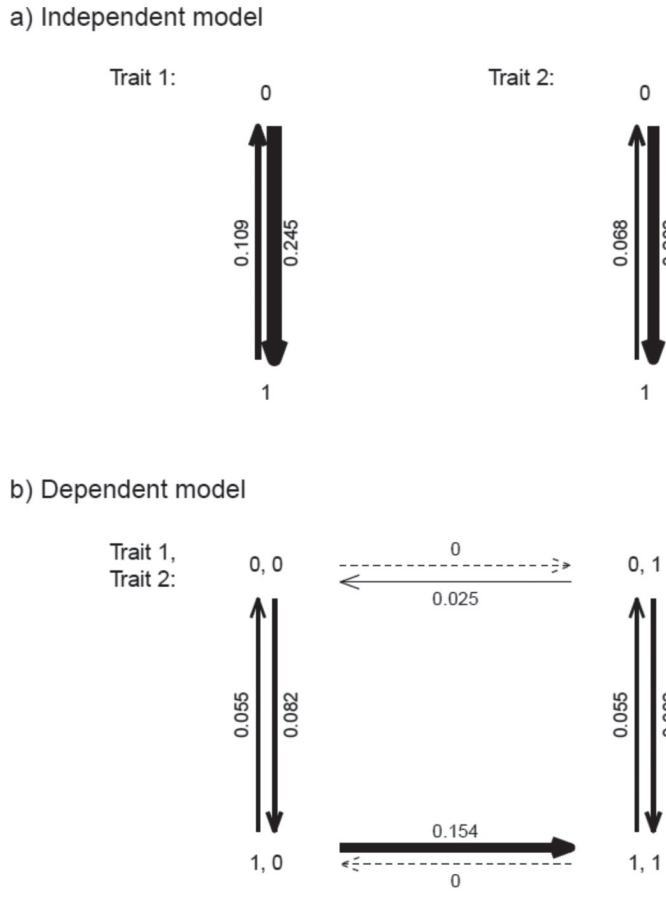

Figure S4. Mating system depends on female-limited colour polymorphism. Graphical representation of the transition rates between traits (females colour polymorphism and mating system) for the independent and the dependent models. Arrow widths are scaled to their average transition rates in that state.