

# Adult insect personality in the wild – *Calopteryx splendens* as a model for field studies

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## Abstract

Animal personality has received increasing interest and acknowledgement within ecological research over the past two decades. However, some areas are still poorly studied and need to be developed. For instance, field studies focused on invertebrates are currently highly underrepresented in the literature. More studies including a wider variety of traits measured and species tested is needed to improve our understanding of trait-correlation patterns and generalities. We studied nine behavioural traits, in the damselfly *Calopteryx splendens*, from an array of three experiments: (i) courtship, (ii) aggressiveness and (iii) boldness, and calculated their repeatability. The behaviours were measured twice, in two different contexts: (i) undisturbed territory and (ii) partially deteriorated territory. All behavioural traits measured, except for two, were repeatable across the two contexts. This work demonstrates, for the first time, the presence of within population personality differences in an adult damselfly in the wild. We further propose *Calopteryx splendens* as a promising model species for testing personality in the wild under highly controlled environmental conditions.

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## Abstract

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personality differences in an adult damselfly in the wild. We further propose *Calopteryx splendens* as a promising model species for testing personality in the wild under highly controlled environmental conditions.

**Key words:** personality, field experiments, studies in the wild, *Calopteryx splendens*, repeatability

## Introduction

Animal personality, defined as inter-individual consistent differences in behaviour across time and context has received growing interest in the past two decades (e.g. Roche et al., 2016). Personality studies have important ecological and evolutionary implications determining such aspects as: space use, species geographic distributions, tendencies to be invasive, response to environmental change, speciation rates, social interactions and fitness consequences (e.g. Briffa and Weiss, 2010; Lichtenstein et al., 2017, 2017; Sih et al., 2004). Repeatability is a highly informative metric that provides a standardized estimate of consistency of individuality, that is, personality (Roche et al., 2016). However, several aspects of animal personality research currently face intense criticism. For instance, there is a underrepresentation of field studies compared to lab-based tests (Archard and Braithwaite, 2010). Also, the amount of studies on invertebrate personality is drastically disproportionate to the number of species and behavioural traits (e.g. Kralj-Fišer and Schuett, 2014). Further, most studies concerns the “Big Five” of animal personality (boldness, aggressiveness, sociability, exploration and activity), ignoring other traits that may bring in-depth understanding of the phenomena and the possible associations between commonly and rarely measured traits (Koski, 2014). Finally, metrics of the personality traits should be chosen with caution in order to be applicable for a given study organism and to represent ecologically relevant information (Carter et al., 2013).

Compared with the large number of laboratory experiments on captive and captive-bred animals, few studies have focused on personality in the wild (Archard and Braithwaite, 2010; Carere and Maestripieri, 2013; Fisher et al., 2015b; Hertel et al., 2020). This skew is unfortunate since lab-based experiments often are affected by a number of constraints: e.g. captivity stress, selective trapping, learning, homogeneity of the laboratory environment, artificial and relaxed selection and reduced pool of potential mates (reviewed by Archard and Braithwaite, 2010). As a consequence, individuals in laboratory conditions may behave in ways not representative for the natural environment and hence showing ecologically irrelevant behavioural patterns (Niemelä and Dingemanse, 2014).

Because of this potential for ecologically irrelevant behavioural patterns in the lab it is important to validate the relevance of findings in the lab with corresponding field studies. Studies designed to compare lab-based and field-based assessments of personality show different results, some fail to find any correlations between lab and field behaviour whereas others provide similar personality estimates in the two environments. For example, studies on crickets showed repeatability of exploration and activity both in laboratory and natural conditions. However, shyness was repeatable in artificial conditions only (Fisher et al., 2015b). Study on zebra finches showed personality both in laboratory and field conditions, but there was no correlation between the two situations (McCowan et al., 2015). In a recent example on sea anemones, Osborn and Briffa (2017) showed that the transition from field to laboratory environment might influence personality assessments. Results from this kind of transplant experiments can therefore be biased by the translocation itself (Niemelä and Dingemanse, 2017). On the other hand, studies on great tits (Cole and Quinn, 2012) or striped mice (Yuen et al., 2016) showed that individuals behaved consistently both in the laboratory and field. The above examples denote that well-designed assessment of personality in a model organism measured in natural field conditions may be of high applicability for understanding the ecological and evolutionary consequences of animal personality (Wolf and Weissing, 2012) in natural populations (Archard and Braithwaite, 2010; Osborn and Briffa, 2017).

Despite the fact that invertebrates represent the most numerous group of animals on Earth (Larsen et al., 2017; Stork, 2018), personality studies on this taxa are still scarce when compared to studies on vertebrates (Gosling, 2001; Kralj-Fišer and Schuett, 2014; Mather and Logue, 2013). However, in recent years insects started to play an important role in animal behavioural research (Keiser et al., 2018). This is because insects’ sexual and social behaviours represent a great variety of phenomena that are rare or absent in

vertebrates, providing new possibilities for addressing ecological or evolutionary questions on personality causes and consequences (Carere and Maestripieri, 2013). Also studies on insects in many instances are less ethically controversial and less time consuming because of a shorter lifespan when compared to vertebrates (Córdoba-Aguilar et al., 2018). However, studies on insect personality in natural conditions without handling and captivity trauma are rare (e.g. Fisher et al., 2015b).

Beyond ‘The big five’ (boldness, aggressiveness, exploration, activity and sociability) which became the blueprint for animal personality studies (Mather and Logue, 2013; Réale et al., 2007; Van Oers and Naguib, 2013), we have limited understanding of other personality aspects. For example, behaviours related to mating have an extraordinary role in species ecology and evolution, but these behavioural traits have received relatively little attention in animal personality studies (Koski, 2014). For instance, The term ‘sociability’ is being used as a proxy for a whole range of behaviours. These include: hiding in presence of a conspecifics’ smell (Cote et al., 2008), grooming in chimpanzees (Koski, 2011), aggregation at food sources in fruit flies (Scott et al., 2018), tendency to shoal in mosquitofish (Brodin et al., 2019) and mating behaviours (Sih et al., 2014). It is possible that the same test/metric may actually measure different traits in two different species (Koski, 2014). For instance, testing response to a predator (as a proxy of boldness) in open areas, which is used for e.g. kangaroos (Blumstein and Daniel, 2003), may not be adequate for a passerine, which usually inhabits, and are preyed upon, in more closed habitats (Whittingham et al., 2004). Also, multiple tests of one personality trait, could be of higher validity in describing a given trait (Carter et al., 2013), as has been done on guppies (*Poecilia reticulata*) where boldness was measured in three experiments (Burns, 2008).

Already established model organisms (i.e., non-human species representing a larger group of organisms used for comparative and integrative research on specific scientific problems, Leonelli and Ankeny, 2013), intensively bred and studied under lab-conditions over several decades, have their limitations and may not be very useful as models for some lines of research. For instance, one of the most significant model organisms, the fruitfly (*Drosophila melanogaster*), intensively used for testing molecular mechanisms of behaviour (Kain et al., 2012; Roberts, 2006; Sokolowski, 2001), has been reared for many generations in homogeneous, non-natural, environments of molecular biology laboratories. This have more than likely resulted in the species adaptation to stable environments and a change in its behavioural reaction to novel conditions when compared to natural populations (Archard and Braithwaite, 2010). For example, a recent study of how anxiolytic pharmaceuticals can affect zebrafish behaviour showed that the behaviour of wild zebrafish was changed by the exposure to the anxiolytic, while the lab-reared zebrafish was unaffected (Vossen et al., 2020). Hence, to increase the ecological relevance of studies including behavioural traits we need to both expand the number and taxonomic breadth of model organisms, as well as re-stock or replace existing lab-populations (Behringer et al., 2009; Leonelli and Ankeny, 2013).

Here, we report a set of behavioural traits tested for their repeatability over time and contexts in the damselfly *Calopteryx splendens* measured in natural field conditions. We measured traits related to three behavioural axes: (i) courtship behaviour, (ii) aggressiveness and (iii) boldness. Since this is the first study on *C. splendens* personality in the wild, we test three traits within each behavioural axis to make sure they are applicable to this study system (Carter et al., 2013). The repeatability was assessed in two different contexts: on undisturbed original patches (males’ territories) and after partial deterioration of a territory. Our results indicate that *C. splendens* is an excellent model for studies on animal personality and behavioural syndromes in nature.

## Methods

### *Study species*

Dragonflies and damselflies are considered as prime model systems for evolutionary and ecological research (Córdoba-Aguilar 2008). One of the most intensively studied families within Odonata is *Calopterygidae* (Córdoba-Aguilar, 2008). *Calopteryx splendens* is a very conspicuous representative from this damselfly family inhabiting lowland rivers in Europe (Askew, 1988; Dijkstra, 2006). *C. splendens* exhibits sexual dimorphism in body colouration, with blue reflecting dark wing spots in the middle of the wings of males, which is a trait

easily recognizable from distance (Askew, 2004). Average life span of a mature male is ca. 1 week (Svensson et al., 2006; Svensson and Friberg, 2007; Tynkkynen et al., 2009) and the territorial and sexual behaviours of calopterygids, as well as traits determining *C. splendens* flight abilities, have been intensively studied over the past 20 years (Marden, 2008; Suhonen et al., 2008). *Calopteryx* sp. males defend territories (e.g. floating aquatic vegetation) and demonstrate a wide range of easily observed behaviours such as patrolling, aerial contests with conspecifics and courtship dances (Golab et al., 2017; Marden and Waage, 1990; Rüppell et al., 2005; Waage, 1973). The flying patterns of different behaviours are very specific and easy to observe with the naked eye from a distance of several meters (Corbet, 1999; Golab et al., 2017; Pajunen, 1966). The species easily habituates to the observer and after a disturbance in/of their environment, damselflies resume normal activity within minutes (Golab et al., 2017, 2013). The adult damselfly and its breeding sites are easily accessible to the investigator (Córdoba-Aguilar & Cordero-Rivera 2005) and breeding site features can be manipulated or highly controlled (Golab et al., 2017, 2013). Trapping and behavioural observations do not affect individuals' behaviours (Golab et al., 2017, 2013) and methods of individual marking and field observations are well established and do not have overt effects on individuals (e.g. Golab et al., 2017; Kuitunen et al., 2010; Plaistow and Siva-Jothy, 1996). Adult *C. splendens* individuals have strong site fidelity and less than 15% of population disperse more than 150 m (Schutte et al., 1997; Stettmer, 1996).

### *Study site and experimental set-up*

Experiments were conducted between 15 June and 31 July 2015 and 2019 on a 50m section of river Biała Nida (Fig. 1), in southern Poland. To reduce the possible influence of weather on damselfly behaviours, the studies were performed during warm, dry and not windy weather (Golab et al., 2013; Tynkkynen et al., 2004). Both the riparian and floating vegetation was prepared for the experiments so that the composition and the spatial structure was homogenous. The size of all floating vegetation rafts (patches), that are defended by males as their territories and used by females as oviposition sites, was similar/comparable among patches (details about the study site and preparation of the river vegetation is described in Golab et al., 2013, Fig. 1). These conditions minimised any microclimate differences between the studied territories. Also, predation in the study area was controlled/limited to a minimum. The only *C. splendens* predators at the study river section were birds, which were not hunting damselflies during the study due to the constant presence of 2-3 observers in the studied river section. Amphibians are other possible predators, but they were not recorded at the study area (Golab and Sniegula, pers. obs.).

Data collections were run between 1000 and 1600 h CEST. First, all *C. splendens* individuals present at the studied section of the river were caught with entomological hand-net and individually marked with a three-digit numbers (white marking pen). Then, five randomly chosen mature males and five females were caught and glued to the fishing-line (Tynkkynen et al., 2008, Fig. 2), placed in a cool-box to prevent energy expenditure, and stored until experiment begins. Experimental bouts were preceded by a 10 min observation of studied territories (patches) in order to assign resident males to their territories. The age of males was assigned to 4 age categories (1 - immature, 2- mature with soft wings, 3- mature without visible wing wear, and 4-mature with some wing damages; Golab pers. obs.). Only males from category 3 were chosen for further studies, since the age can influence male territorial behaviour (Corbet, 1999; Tynkkynen et al., 2009) and could influence a males response to our experimental treatments.

For each resident male three types of experiments were run: (1) courtship experiment – female attached to a fishing line was presented to a focal resident male for 2 min, (2) aggressiveness – a male attached to a fishing line was presented to a resident male for 2 min and (3) boldness – a bird decoy was moved toward a resident male (simulated predator attack) until he flew away and latency to return to his territory was measured (Tab. 1). In every experiment we measured three traits (described in Tab. 1). Each experiment was run twice: on the original patches (morning trial) and repeated on the patches manipulated by sinking ca. 25% of each vegetation patch using a ballast (afternoon trial) (Fig. 1B). The minimum time between the two rounds of the experiment was 1 hour. Males and females on the fishing line were replaced by new ones every 20 min, in order to avoid exhaustion or rejection signals in case of females (Tynkkynen et al., 2008). The patch manipulation aimed at measuring the traits across two situations and times (Dingemans

and Reale, 2005; Sih et al., 2004).

### Statistical analyses

We assessed behavioural consistency (i.e., personality) by quantifying repeatability coefficient for the nine traits using the package ‘rptR in R v.4.0.2. (R Development Core Team, 2016; Stoffel et al., 2017). Repeatability coefficient (R) calculated as ratio of group level variance over the sum of group level and residual variance gives the information about how particular trait correlates between replication in one replication unit (in our case individual). The coefficient takes value between 0 and 1. In rpt models we set the bootstrap number to 1000 in order to properly estimate confidence intervals for repeatability coefficient.

## Results

The estimated population density, assessed based on a standardised daily mark-release procedure, was ca. 1 individual per 1 m section of the river, which is an intermediate density for the species (Chaput-Bardy et al., 2010; Kuitunen et al., 2010; Stettmer, 1996).

All behavioural traits related to courtship and boldness were significantly repeatable. In contrast, among the traits connected to aggression only “number of hits” was repeatable (Tab. 2).

## Discussion

We report for the first time personality in a natural population of the damselfly *C. splendens* measured in the wild. We show cross-context repeatability in most of the traits studied. Traits related to both courtship and boldness axes showed repeatability values close to the average value of behavioural repeatability across over 750 studies of various behavioural traits and taxa (Bell et al., 2009). Our research responds to the need to study personality in natural field conditions in order to assess ecologically relevant situations and contexts (e.g. Archard and Braithwaite, 2010; Hertel et al., 2020). Further, our study indicates that *C. splendens* is likely suitable to become a model organism in behavioural studies.

Generally, individuals’ latency to approach a rival and number of bites are one of the most commonly used indicators of aggressiveness and show high repeatability in most studies (Keiser et al., 2018). In our case, absence or low repeatability for both reaction to intruder and number of bites (Tab.2), are in contrast to that trend, and are also in contrast to an earlier meta-analysis (Bell et al., 2009). However, in crickets, Fitzsimmons and Bertram (2013) showed low repeatability of aggression scores (quantified from the duration and frequency of agonistic behaviours during contest). The authors suggested that the trait plasticity was an effect of social environment as well as the insect physiology (Fitzsimmons and Bertram, 2013). In our study, the intruder male was chosen randomly, and we did not take measures of their physiological condition, which might have been useful for a deeper understanding of our result. Also, we propose that future studies should be using a mirror (e.g. Balzarini et al., 2014) instead of an actual rival, and that this would bring more controlled and comparable metrics of aggression. In the case of banded demoiselle, the time needed for reaction to rival male might also be strongly influenced by the social environment (Bell et al., 2009). In this species antagonistic behaviours depend on whether the potential rival male is a neighbour, a wandering male or an actual opponent. It has been shown that neighbouring territorial males avoid contest (Briffa and Weiss, 2010; Golab et al., 2017; Gordon, 1997; Maynard Smith and Parker, 1976). Also, non-territorial *C. splendens* males show a characteristic wandering behaviour, that is, patrol larger sections of a river looking for territories or mates. During this activity non-territorial males may either pass a given territory, approach the resident and retreat immediately or approach and initiate a conflict of varying intensity (Koskimäki et al., 2009; Panov and Opaev, 2013). Resident males have to evaluate which of the three type of males he is facing for every interaction and react adequately to the situation.

With regard to biting an intruder, based on our data and earlier studies, we suggest that this trait probably is unsuitable for personality measures. The biting behaviour requires a situation when the head (mandible) of the resident male approaches the part of the opponent’s body that is ‘suitable for biting’. In aerial contest (Marden and Waage, 1990) biting the intruder may arise by chance, depending of the direction/intensity/frequency of the chasing damselflies movements. Also, some parts of the body may simply be

harder to bite, for instance the centre of a wing area. Additionally, despite the fact that dragonflies have one of the most advanced visual systems among insects (Bybee et al., 2012) and can compute flight trajectory of their prey (Olberg, 2012) there is no evidence that odonates would be able to compute their opponents body movements during fighting in order to precisely bite one another. Summarizing the above, we conclude that the time for reaction to the opponent and number of bites in *C. splendens* are plastic traits, depending on either social, environmental or physical factors, or a combination of them all and hence are not useful for personality studies in calopterygids.

In contrast, the number of hits seemed to be a better proxy of aggression in *C. splendens* in the wild. Generally, *Calopteryx* spp. males compete for resources during aerial contests (Marden and Waage, 1990), but most of the disputes are brief pursuit flights after which an intruder is chased away. During escalated longer aerial fights some collision or hitting can occur (Rüppell et al., 2005; Golab pers. observ.). The above discussed results illustrates the importance of choosing the right test for estimating a personality trait (Sih and Bell, 2008) and developing multiple proxies for a given behavioural axis might be crucial to identify the most suitable test for the targeted trait in a given species (Carter et al., 2013).

The time a resident male needed to react to an approaching female showed individual consistency (Tab. 2). The trait may appear similar to the reaction to an intruder, which was not repeatable, but when reacting to a female the resident male wants to attract rather than chase away (Corbet, 1999). In addition, the two traits related to courtship: dive display and engagement were also consistent and, as such, potentially a good metric for personality. Consistent engagement to mating displays has also been shown for instance in male guppies (Biro et al., 2016; Magellan and Magurran, 2007). This is all in accordance with theory that predicts consistency in mating behaviours since it reduces cognitive costs for potential mates (Dall et al., 2004). Also, given that personality traits are heritable (Korsten et al., 2013; Réale et al., 2007) and the offspring environment is predictable, female (choosing partner) can assess what behavioural traits would be adaptive for her offspring and choose a sexual partner of a beneficial behavioural profile. But on the other hand, highly variable or unpredictable environments would favour behavioural plasticity rather than consistency (Dingemanse et al., 2010). Hence, there are studies showing no personality in mating-related behaviours as for example research on subordinate reindeers, whose propensity to enter/visit mating group is based on proximate factors such as the group sex ratio and a day of mating season (Strong, 2015).

Among insects one of the most advanced personality research in the wild has been conducted on crickets. In an interesting and large-scale project “Wild Crickets” (<https://www.wildcrickets.org/>) a group of researchers studied personality both in the field and in the laboratory. They found that individual behavioural consistency is steady over adult lifetimes (Fisher et al., 2015a) and that personality in captivity not always predicts personality in nature (Fisher et al., 2015b). This is in line with another study on crickets *Gryllus campestris* showing that handling procedure in translocation experiments may bias repeatability estimates (Niemelä and Dingemanse, 2017). In our study we did not compare field with lab-based experiments. However, since there is growing evidence that gene expression can be significantly modified by environmental factors (Niemelä and Dingemanse, 2014) and artificial conditions can impose additional unnatural stressors (Archard and Braithwaite, 2010), we conclude that field studies are superior, in ecological relevance, compared to lab-based experiments on most animals including adult calopterygid damselflies. More specifically, the methods presented in this study are particularly promising for studying adult damselfly behaviour since they reduced handling trauma, did not influence natural/free behaviour of damselflies during the observations (Golab – pers. obs.) and prevented damselflies from adjusting/habituating to the procedure (Archard and Braithwaite, 2010; Hilfert-Rüppell, 1999).

One common difficulty when studying animal personality in the field is controlling environmental heterogeneity (Bell, 2004; Dingemanse and Réale, 2013; Quinn et al., 2009). In our experiments environmental factors were natural and many were standardized for: microclimate (sunlight penetration, air temperature, wind speed), water quality (current, temperature velocity), composition and structure of macrophytes (Gibbons and Pain, 1992; Guillermo-Ferreira and Del-Claro, 2011; Hilfert and Ruppell, 1997; Hilfert-Rüppell, 1999; Siva-Jothy et al., 1995, 1995; Siva-Jothy and Hooper, 1995) and predation (Golab and Sniegula, pers.

obs., details in methods). This adds a robustness to our results that often can be lacking in animal personality field-studies due to varying environmental conditions.

Here we emphasise that *Calopteryx* spp. express other ecologically important behaviours, beyond the “Big Five”(Keiser et al., 2018), that could be potentially useful for future personality studies (Koski, 2014). These traits include: territory patrolling (Corbet, 2004; Golab et al., 2017), gathering of non-territorial males (Golab et al., 2013) and a very elaborated repertoire of courtship behaviours (Corbet, 2004; R  ppell et al., 2005).

To summarize, our work is the first that demonstrate behavioural repeatability in an adult damselfly in the wild. Our results suggest that adult *C. splendens* is a very promising model organism for studying insect personality under ecologically relevant natural conditions. The species has an elaborate repertoire of behaviours that can be easily observed and measured swiftly using only the naked eye. In addition, they also have a strong site fidelity which enables controlled and relevant manipulations of key environmental parameters. We suggest that our study represents the natural variability that exists in studied behaviours of this species. Two of the traits related to aggressiveness were not consistent and should hence not be useful for personality tests or experiments. This emphasises the need of proper trait selection when aiming to understand ecological implications of differences in individual behaviour.

### Data accessibility

Upon accepting the manuscript, the data will be archived in Dryad.

### Competing Interests

The authors declare that they have no conflict of interest.

### Author Contribution

Project design: MJG, SS. Data collection and analysis: MJG, SS, AA. Manuscript writing MJG, SS, TB, AA.

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## Tables

Table 1. Behavioural traits measured in three experiments on *C. splendens* in Biała Nida river.

Experiment	Traits	Description
Courtship	Reaction to female	Time [s] until focal resident moved toward a female
	Dive display	Number of alighting of a resident male on the water surface (a common courtship display in <i>Calopteryx</i> sp.)
	Engagement	A nominal value describing a male devotion to courtship: 100%: male attempts to form a tandem, dives on water, patrols a territory, chases away intruders, does not fly aside (does not leave his territory during the experimental period), does not perch (does not stop flying during the experiment); 75%: male patrols a territory, chases away intruders, does not fly aside, does not perch 50%: male patrols territory, chases away intruders, flies aside, perches; 25%: no patrolling, no chasing, flights aside, perching during the experimental trial
Aggressiveness	Reaction to intruder	Time [s] until focal resident moved toward the presented intruder male
	Bites	How many times a resident male bit the intruder
	Hits	How many times a resident male hit the intruder
Boldness	Distance to react	Binomial distance value: Near - male escaped when the bird decoy was closer than 0.5m Far - male escaped when the bird decoy was more than 0.5m away
	Escape distance	Distance [m; with an accuracy of 0.5m] the focal resident male flew after the predatory attack simulation
	Time to return	Time [s] passed until the resident returned to his territory after the predatory attack. The maximum time the observer waited for the male to come back was 180 s

Table. 2. Repeatability (R) of behavioural traits of *C. splendens* in Biała Nida river.

Trait	R	P	95% CI
Reaction to female	0.396	< <b>0.001</b>	0.174, 0.553
Dive display	0.338	< <b>0.001</b>	0.184, 0.465
Engagement	0.492	< <b>0.001</b>	0.339, 0.624
Reaction to intruder	0.167	0.055	0, 0.332
Bites	0.160	0.109	0, 0.347
Hits	0.364	< <b>0.001</b>	0.113, 0.541
Distance to react	0.396	< <b>0.001</b>	0.174, 0.553
Escape distance	0.338	< <b>0.001</b>	0.161, 0.491
Time to return	0.282	<b>0.001</b>	0.098, 0.440

## Figure legends

Figure 1. Study site in Biała Nida river including selected vegetation patches. Six patches of floating vegetation (*Potamogeton natans*) manipulated to equal size, used as territories by males and oviposition substrate by females (A). Sequential experimental patch area reduction using a ballast (B).

Figure 2. Experiment in the field. A – *C. splendens* female glued to a fishing line; B – Flying *C. splendens* male glued to a fishing line.

