Hybridization between *Ischnura graellsii* (Vander Linder) and *I. elegans* (Rambur) (Odonata: Coenagrionidae): are they different species?

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Two closely related damselflies, *Ischnura graellsii* and *I. elegans*, were analysed for morphological differences and reproductive isolation in the north coast of Galicia (NW Spain). We compared animals from sympatric and allopatric localities, including *I. elegans* from Belgium and *I. graellsii* from southern Spain as pure allopatric populations. A set of morphometric characters were studied by means of multivariate discriminant analysis to determine if these two species can be unambiguously distinguished. Discriminant analysis revealed that *I. graellsii* and *I. elegans* are well differentiated on the first two axis (86% and 11%, respectively). *I. graellsii* individuals are distinguished from *I. elegans* by their smaller size and, specifically, by their narrower and shorter wings and shorter tibiae. In addition, *I. elegans* has a narrower space between the branches of each cercus, and greater distance between the branches of each paraproct. Sympatric individuals are morphologically intermediate, suggesting hybridization. When the species were put together in the laboratory, they showed partial temporal separation in mating behaviour, but males of *I. elegans* readily mated with females of *I. graellsii*, and hybrid individuals were obtained. The opposite heterospecific cross was almost impossible, apparently because of mechanical problems with the tandem linkage. Laboratory-reared hybrids (from male *I. elegans* ¥ female *I. graellsii*) are morphologically intermediate, mainly resembling the maternal phenotype. Although hybridization between both taxa is common, we suggest maintaining the specific status for both phenotypes because they show incipient reproductive isolation, as it is reported in the literature. © 2002 The Linnean Society of London, Biological Journal of the Linnean Society, 2002, 76, 225–235.


INTRODUCTION

Natural hybridization is a common process in plants, but in most groups of animals, diversification over time is interpreted as a series of branching events, in which hybridization occurs rarely (Harrison, 1993). In some cases, a hybrid zone could arise as a consequence of secondary contact between two populations that have evolved in sympatry (Barton & Hewitt, 1989). However, in many other cases, one of the two species, or even the hybrid, becomes more successful and displaces one or both of the original taxa (Mallet, 1986; Harrison, 1993; Szimura, 1993; Carmona et al., 1997; Brede et al., 2000; Klingenber, Spence & Mirth, 2000).

Odonates (damselflies and dragonflies) are ideal models to test hypotheses about reproductive isolation because their reproductive behaviour is unique among animals: the male intromittent organ is situated on the second abdominal segment, whereas the female has the vagina at the end of the abdomen. Therefore, males must first grasp the female by her prothorax (or head) with their anal appendages to copulate. Thus, morphological incompatibility can be an important barrier in odonates whose male appendages differ substantially between species while female coloration does not (Paulson, 1974; but see also Robertson & Paterson, 1982). Hybridization seems uncommon in odonates (Tennessen, 1982), but some cases of puta-
tive intrageneric hybrids have been reported (Corbet, 1999). To our knowledge, the only certain hybrids so far obtained among odonates are hybrids between *Ischnura gemina* and *I. denticollis* (Leong & Hafernik, 1992a).

In this study, we analyse morphological differences between two closely related damselflies, *Ischnura graellsii* and *I. elegans*, whose genetic distance is 0.196%, based on 1058 bases of cytochrome *b* and coenzyme II combined (J. V. Robinson, pers. comm. 2001). These species are very similar, but they can be separated by looking at the prothoracic tubercle and the morphology of the terminal abdominal appendages (Askew, 1988). They live in the same habitats, including ponds, wetlands, coastal lagoons, salt marshes or even slowly flowing rivers, but nevertheless are very rarely found in sympathy, suggesting extreme competition (Compte Sart, 1988). Jödicke (1996a) found sympatric populations in NE Spain, and a few intermediate individuals that he described as putative hybrids. We discovered that the distribution of *I. elegans* and *I. graellsii* overlaps in the north coast of Galicia (NW Spain), and that many intermediate individuals were found at one location, suggesting hybridization.

Our first aim was to test if both species can be unambiguously distinguished by simple morphometrics. Furthermore, we present the results of laboratory experiments to test for precopulatory reproductive barriers. We have reared laboratory hybrids and compared them with natural putative hybrids, and discuss some hypotheses to explain the partial overlap in the distributions of these species.

**MATERIAL AND METHODS**

**DISTRIBUTION**

We searched the literature for records of both species in the Iberian Peninsula, contacted odonatologists for unpublished information, and plotted all known localities using DMAP® 7.0e software (http://www.dmap.co.uk/). Most data were obtained in general sampling of odonates; therefore, the absence of one species from a particular area is not due to a lack of sampling. The resulting map is based on 437 records for *I. graellsii* and 151 for *I. elegans*. The main references are Ocharán (1987), Compte Sart (1988) and Jödicke (1996b).

**MORPHOMETRICS**

Four allopatric populations of *I. elegans* and three allopatric populations of *I. graellsii* were sampled at 10 different localities in Galicia (NW Spain), in 1990 and 2000 (Table 1). We found both species at the Foz locality, and also some intermediate individuals which we suspect are natural hybrids. In addition, two allopatric populations situated far from those cited above were sampled in 2001, in the Southern region of Spain (Córdoba) for *I. graellsii* and in Antwerp (Belgium) for *I. elegans*. Hybrid males obtained and reared from crosses between virgin females and males were also measured and the data added to the matrix.

To record morphometric data, 30 males were selected at random from each allopatric sample, except for Córdoba and Antwerp, where 23 and 27 individuals were sampled, respectively. In Foz, samples were represented by 19 putative male hybrids identified as *I. graellsii* and 13 as *I. elegans*. Eleven continuous characters and two count characters were measured, representing major body regions: head, thorax, wings and anal appendages (Table 2, Fig. 2). Individual body parts were observed by binocular microscope, digitized and measured with the software Global Lab 3.0®. We studied the anal appendages that are used by

<table>
<thead>
<tr>
<th>Locality</th>
<th>UTM coordinates</th>
<th>N</th>
<th>Species</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xuño</td>
<td>29TMH9620</td>
<td>30</td>
<td><em>I. graellsii</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>A Lanzada</td>
<td>29TNH1000</td>
<td>30</td>
<td><em>I. graellsii</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Corrubedo</td>
<td>29TMH9514</td>
<td>30</td>
<td><em>I. graellsii</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Córdoba (S. Spain)</td>
<td>31SFB5398</td>
<td>23</td>
<td><em>I. graellsii</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Pantín</td>
<td>29TNJ7132</td>
<td>30</td>
<td><em>I. elegans</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Louro</td>
<td>29TMH9234</td>
<td>30</td>
<td><em>I. elegans</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Doniños</td>
<td>29TNJ5515</td>
<td>30</td>
<td><em>I. elegans</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Valdoviño</td>
<td>29TNJ6828</td>
<td>30</td>
<td><em>I. elegans</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Antwerp (Belgium)</td>
<td>31UES9363</td>
<td>27</td>
<td><em>I. elegans</em></td>
<td>Allopatric</td>
</tr>
<tr>
<td>Foz</td>
<td>29TPJ4123</td>
<td>19 + 13</td>
<td><em>I. graellsii + I. elegans</em></td>
<td>Sympatric</td>
</tr>
</tbody>
</table>

males to grasp the female’s prothorax during mating because their shape is important in species identification (Aguesse, 1968; Askew, 1988). To characterize the anal appendages we measured the distance between the tip of the appendages of the same pair, and also the angle formed between three points: the vortex was the tip of one paraproct, one of the end points was the tip of the cercus of the same side, and the other was the tip of the opposite paraproct (Fig. 3E). Right and left angles were measured. We estimated measurement error by taking three measurements (on three different days) of all variables in 30 individuals from the Doniños locality (Table 2). The proportion of variance due to measurement error was estimated from a one-way ANOVA with individuals as the factor variable, using BIOMstat® ver. 3.2 (Rohlf & Slice, 1997). Measurement error was in general low (0–10%), but four variables (thorax length, head width, head length and wing width) had a larger error (19–38%).

We obtained morphometric measurements from a total number of 292 field-collected males and 11 laboratory males, reared from larvae. Data were analysed by a discriminant analysis function, using xlSTAT® 4.0 software (http://www.xlstat.com). Populations of *I. graellsii* and *I. elegans* in allopatry were used as reference groups, as were hybrids obtained in the laboratory. Sympatric individuals were classified a priori following Askew (1988), and they were introduced into the analysis, once the discriminant function was calculated from the reference groups, to obtain the cross validation.

## Laboratory Hybridization and Mating Behaviour

In a set of laboratory experiments, virgin females and males were maintained in breeding cages following Cordero (1990), and observed from 08:00 to 20:00 hours, at 27–29°C, 60–80% RH, and a natural photoperiod of 15:9 h light: dark. Density in each cage was five or six individuals from each species and sex. During this 12-h period, specimens were observed every 10 min. For each mating, the identity of individuals, time of tandem beginning and total duration of mating were recorded. Copulation duration distributions were compared between crosses by means of a Kolmogorov–Smirnov non-parametric test ($\alpha = 0.05$). This test is sensitive to any kind of difference in distributions from which the two compared samples were drawn (Siegel & Castellan, 1988).

## Results

### Distribution

We found that both species have an overlapping distribution on the Mediterranean coast and central-eastern Spain (Fig. 1). *I. graellsii* is conspicuously absent from the Balearic islands and the NW coast of Galicia, and *I. elegans* from Portugal, SW Spain and the north coast of Spain (except NW) (see also D’Aguilar, Dommanget & Préchac, 1998). There are no samples from North Portugal, but *I. graellsii* should be common there. Our review of the literature and personal observations revealed that both species were found cohabiting the same habitat only in 16 localities. However, when the same localities were revisited, samplings showed that six of these habitats had only *I. elegans*, but none had only *I. graellsii*.

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**Table 2.** Measurement error (%, ME), canonical vector coefficients for the two first axis (CV1 and CV2) and correlations between the values of the discriminant function and the values of variables ($r_1$ and $r_2$). See Fig. 2 for definition of morphometric variables.

<table>
<thead>
<tr>
<th>Character</th>
<th>ME</th>
<th>CV1</th>
<th>CV2</th>
<th>$r_1$</th>
<th>$r_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax length (ThL)</td>
<td>18.9</td>
<td>-0.306</td>
<td>0.824</td>
<td>0.409</td>
<td>-0.153</td>
</tr>
<tr>
<td>Thorax depth (ThD)</td>
<td>6.0</td>
<td>-0.490</td>
<td>2.091</td>
<td>0.462</td>
<td>-0.05</td>
</tr>
<tr>
<td>Head width (HW)</td>
<td>38.5</td>
<td>-1.156</td>
<td>0.377</td>
<td>0.327</td>
<td>-0.228</td>
</tr>
<tr>
<td>Head length (HL)</td>
<td>26.7</td>
<td>-0.265</td>
<td>1.377</td>
<td>-0.028</td>
<td>-0.037</td>
</tr>
<tr>
<td>Wing length (WL)</td>
<td>1.6</td>
<td>0.614</td>
<td>-1.535</td>
<td>0.873</td>
<td>-0.382</td>
</tr>
<tr>
<td>Wing width (WW)</td>
<td>19.7</td>
<td>2.194</td>
<td>-2.183</td>
<td>0.808</td>
<td>-0.395</td>
</tr>
<tr>
<td>Number of antenodal cells in right forewing (NC)</td>
<td>0.0</td>
<td>-0.075</td>
<td>0.469</td>
<td>0.545</td>
<td>0.112</td>
</tr>
<tr>
<td>Right fore-tibia length (TL)</td>
<td>10.3</td>
<td>0.667</td>
<td>0.287</td>
<td>0.668</td>
<td>0.041</td>
</tr>
<tr>
<td>Number of spines in right fore-tibia (NS)</td>
<td>7.4</td>
<td>-0.004</td>
<td>0.018</td>
<td>0.371</td>
<td>0.061</td>
</tr>
<tr>
<td>Left angle of anal appendages (LA)</td>
<td>10.7</td>
<td>-0.023</td>
<td>-0.036</td>
<td>-0.738</td>
<td>-0.335</td>
</tr>
<tr>
<td>Right angle of anal appendages (RA)</td>
<td>11.9</td>
<td>-0.008</td>
<td>0.0002</td>
<td>-0.678</td>
<td>-0.174</td>
</tr>
<tr>
<td>Distance between cerci (DC)</td>
<td>1.5</td>
<td>-1.268</td>
<td>-0.225</td>
<td>-0.584</td>
<td>-0.006</td>
</tr>
<tr>
<td>Distance between paraprocts (DP)</td>
<td>4.9</td>
<td>3.954</td>
<td>4.379</td>
<td>0.907</td>
<td>0.296</td>
</tr>
</tbody>
</table>
Laboratory hybridization and mating behaviour

In the laboratory, precopulatory reproductive isolation was incomplete. Male *I. elegans* easily mated with female *I. graellsii*, but the opposite cross was never obtained, in agreement with preliminary results (Cordero, 1989). We suspect that tandem between male *I. graellsii* and female *I. elegans* is impeded by the prothoracic tubercle of *I. elegans*, which is larger.
for this latter species, in both females and males (see Fig. 2 for males). In relation to mating behaviour, when the two species were set together in the same cage from 08:00 hours, only *I. elegans* males started to copulate early in the morning, with either *I. elegans* or *I. graellsii* females. Intraspecific copulations of *I. elegans* occurred from 08:55 to 16:40 hours, and all of them finished by 18:45 hours. Intraspecific matings of *I. graellsii* started later, ranging from 11:10 to 19:55 hours, the last mating finishing beyond 20:30 hours. Only one cross (out of 22) started earlier than 11:00 hours, at 9:10 hours. The duration of interspecific crosses was probably controlled by *I. elegans* males, since they started early, at 08:53 hours, and lasted until 17:05 hours.

For both species, the earlier they started to copulate the longer the mating took. Duration of copulation was significantly longer for intraspecific crosses of *I. graellsii* than for crosses where *I. elegans* males were copulating with females either of *I. elegans* or *I. graellsii* (Mean ± SE, Ie × Ig and Ie × Ie = 198.68 ± 20.58, N = 39; Mean ± SE, Ig × Ig = 270.85 ± 34.60, N = 22; Z = 1.499; P = 0.022; Fig. 4).

**Morphometric analysis**

Discriminant analyses revealed that individuals of *I. elegans* and *I. graellsii* are well separated on the first and second axes, which accounted for 85% and 11% of the variance, respectively (Fig. 5). Wilk’s lambda
was low (0.0309), which means that most of the total variability is attributable to differences between the means of the groups (Norušis, 1986) (Wilk's test, $P = 0.0001$).

$I. graellsii$ individuals are distinguished from $I. elegans$ by narrower and shorter wings and also shorter tibiae and thorax (in general, smaller size). They are also clearly distinguished by the size of the prothoracic tubercle (Fig. 2). In relation to secondary genital appendages, $I. graellsii$ has a narrower space between the paraproct tips, but a greater distance between the cerci tips and higher angles between both cerci and paraprocts (Fig. 3).

Although discriminant analyses recognize non-Galician individuals as different groups, they are mainly discriminated in the second axis from those individuals captured in Galicia (Fig. 5). These individuals have higher values for WL, WW and for LA and RA (Table 2) in abdominal appendages than their conspecifics from Galicia (in general larger size). However, distances of Mahalanobis (Norušis, 1986) between individuals sampled out of Galicia and conspecific individuals from Galicia are smaller than distances between both species in Galicia ($D_1^2 I. elegans$ Galicia, $I. elegans$ Antwerp = 11.18; $D_1^2 I. graellsii$ Galicia, $I. graellsii$ Córdoba = 11.31; $D_1^2 I. elegans$ Galicia, $I. graellsii$ Galicia = 38.32). Distance matrix also showed that laboratory hybrids are closer to $I. graellsii$ than to $I. elegans$ ($D_2^2 I. elegans$ Galicia, hybrids = 21.16; $D_2^2 I. graellsii$ Galicia, hybrids = 11.06; Fig. 5). Furthermore, 92% of hybrid males had a large tubercle on the prothorax, but smaller than in $I. elegans$ (Fig. 2B,D). However, abdominal appendages were similar to $I. graellsii$ in 75% of cases, since internal processes of the cerci are frequently crossed and tips are more distant than in $I. elegans$ abdominal appendages (Fig. 3E,F).

Correlations between values of the discriminant function and the variables showed that the best variables for discrimination (those with high correlations) were DP, WL and WW (Table 2). LA, RA and TL had the next highest values.

Fifteen cases were misclassified. Of these, seven individuals were moved from $I. elegans$ Galicia to $I. elegans$ Antwerp, which demonstrates that there are no conspicuous differences that separate individuals from both localities and that they are the same species. In the same way, one individual from $I. graellsii$ Córdoba was moved to $I. graellsii$ Galicia, and another one suffered the opposite change. On the other hand, one individual was moved from $I. elegans$ Galicia to $I. graellsii$ Galicia and the rest were $I. graellsii$ and $I. elegans$ individuals that were reclassified by the discriminant analysis as laboratory individuals. Error rate of classification was 0.0554.

Cross validation of sympatric individuals, classified a priori as specimens of $I. graellsii$ or $I. elegans$ from Galicia, indicated that 46.9% of individuals were included in the wrong group. From these, four individuals preclassified as $I. graellsii$ were moved to $I. elegans$ and two individuals were moved in the opposite direction. One individual from $I. elegans$ Galicia was reclassified in $I. elegans$ Antwerp and two individuals of $I. graellsii$ Galicia were included in $I. graellsii$ Córdoba. Finally, there were also five individuals that were preclassified as $I. elegans$ and were reclassified as laboratory hybrids.

Hence, while most laboratory hybrids are similar to maternal phenotype, individuals living in a sympatric situation in the field have a broader distribution, overlapping with both $I. elegans$ and $I. graellsii$ data (Fig. 5).

**DISCUSSION**

Our laboratory experiments suggest that prereproductive isolation between $I. graellsii$ and $I. elegans$ is incomplete. While female $I. elegans$ have a large prothoracic tubercle (see Fig. 2B–D for males; females have the same tubercle morphology) that impedes tandem formation with male $I. graellsii$, the opposite pairing (male $I. elegans \times$ female $I. graellsii$) is nevertheless undistinguishable from conspecific matings, either in duration or in frequency. Our findings concur with those found by Jödicke (1996a), who examined 20 pairs found in copula in NE Spain, where both species live syntopically, and found two pairs of male $I. elegans$ – female $I. graellsii$ and one pair of male $I. graellsii$ – female $I. elegans$. He also found two females and three males which he described as putative hybrids. Similar observations on effective crosses in only one direction were made by Leong & Hafernik (1992a) for $I. denticollis$ and $I. gemina$. In the field, the importance of tandem linkage as an isolation barrier is usually evident after the action of other factors such as habitat, activity, etc. (Tennesen, 1982). In the laboratory, however, the effect of these factors is obviously reduced and, in addition, there is a similarity in reproductive behaviour that enhances the likelihood of heterospecific crossing. It is known that females have mechanoreceptors on the mesostigmal plate that are presumably stimulated by male cerci during tandem linkage (Robertson & Paterson, 1982). Females of $I. elegans$ refuse copulation with conspecific males having experimentally altered appendages (Krieger & Krieger-Loibl, 1958), which is suggestive of a sexual selection mechanism for the evolution of the shape of anal appendages.

Tennesen (1982) classified the prereproductive isolating barriers in Odonata into four main groups: temporal, habitat, ethological and mechanical. He suggested that most odonates might be isolated by ethological barriers, with visual and tactile stimuli as the most important barriers, but also stressed the lack...
Figure 3. Male abdominal appendages in anal view of (A) *I. elegans* and (B) *I. graellsii* from allopatric populations, (C) ‘*I. elegans*’ and (D) ‘*I. graellsii*’ from a sympatric population, and two hybrids obtained in the laboratory (E, F).
Figure 4. Duration of intra- and heterospecific copulations of *I. elegans* and *I. graellsii* bred in the same laboratory environment. Note that *I. elegans* starts and ends copulation earlier than *I. graellsii*. (Ie: *Ischnura elegans*; Ig: *I. graellsii*).

Figure 5. Plot of the first and second canonical variate scores of *I. graellsii* and *I. elegans* from sympatric (Foz) and allopatric populations, and laboratory-reared hybrids. (Ie: *Ischnura elegans*; Ig: *I. graellsii*).
of research on hybridization in this group. The results of this study indicate that *I. elegans* and *I. graellsii* can mate in the laboratory, giving viable offspring. This contrasts with earlier attempts to cross both species which failed because females laid only a few sterile eggs (Cordero, 1989). Although it is considered that odonates do not hybridize frequently, there are some reported cases of heterospecific tandem pairs and hybrids, and this process is apparently more common in Anisoptera than in Zygoptera (Schneider & Krupp, 1996). Some of the reported hybrids belong to *Ischnura* species (Leong & Hafernik, 1992a, b; Jödicke, 1996a; Schneider & Krupp, 1996). This is probably due to the similar reproductive behaviour and morphology of these species, and lack of complex courtship displays and aggressive behaviour (Corbet, 1999). There is even a report of *I. erratica* mating with individuals from other families, such as Lestidae (Paulson & Cannings, 1980). We have also found a male *I. elegans* in copula with a female of a different coenagrionid, *Pyrrhosoma nymphula* (A. Cordero, unpubl. data), and one mating between a female of *I. graellsii* and a male of *I. pumilio* (Cordero, 1992). Owing to similarities in morphology and colour of *I. graellsii* and *I. elegans*, visual recognition seems unlikely to ensure reproductive isolation (Corbet, 1980), so structures involved in tandem and mating might be playing an important role.

Our laboratory experiments showed some temporal separation in mating activity, since *I. elegans* begins copulation in the morning, and does not extend it beyond late afternoon. On the other hand, *I. graellsii* seems to prefer mating beginning early in the afternoon, although time of tandem formation could overlap for some hours with copulation of *I. elegans*. In any case, *I. graellsii* always extend their mating until late in the evening, with a mating duration as long as 9h 19min. Interspecific crosses are governed by *I. elegans* males. Hence, these interspecific matings showed a very similar temporal pattern to *I. elegans* intraspecific crosses. Copulation duration was negatively related to time of start. Thus, for both species, the earlier they started to copulate the longer the mating lasted. This pattern could be interpreted as a male guarding behaviour already seen in other species of Odonates (Cordero, 1990; but see Andrés & Cordero-Rivera, 2000). A temporal separation of mating activity also apparently occurs in nature, since *I. graellsii* is frequently observed copulating late in the afternoon or even in the evening (Cordero, 1989, 1992), while *I. elegans* copulates during the morning or early in the afternoon (Cordero, Santolamazza Carbone & Utzeri, 1998). However, it is also expected that this copulatory activity could vary with climate and latitude, since Gorb (1999) found that *I. elegans* in Jagotin, Ukraine, starts mating activity late in the morning. Complete temporal separation in mating behaviour could impede hybridization, but the separation is not very clear at our study sites.

The idea of the existence of a single, polymorphic species distributed in Iberia is rejected. We suggest that the status of ‘good-species’ should be maintained for the taxa analysed, since both phenotypes are well discriminated with a low error rate. For instance, in the first classification (before adding the sympatric specimens), only one individual out of 292 was changed from the *I. elegans* group to the *I. graellsii* group. Genetic distance clearly indicates that both are very closely related (as semispecies) and a preliminary analysis of mitochondrial DNA suggests that the divergence occurred about 100 000 years ago (J. V. Robinson, pers. comm. 2001). They are therefore incipient species (Carchini et al., 1994).

On the other hand, hybrid individuals obtained in the laboratory were recognized as a distinct group, but they were located near the *I. graellsii* group, and the distance among them was small. However, when cross validation of sympatric individuals was carried out, it showed a high error rate of classification. From these analyses, some interesting conclusions can be gathered. First, there were five individuals that were reclassified as laboratory hybrids, which means that they can actually be field hybrids and that hybridization could occur in the field. In addition, there are individuals that were misclassified as one of the species and they were changed to the other, meaning that individuals that live in sympathy have intermediate characteristics that may confuse their identification. These individuals could have experienced some hybridization or back-crossing processes in previous generations that could have influenced their morphology. Finally, some individuals were changed from Galicia to Antwerp in the case of *I. elegans*, and to Córdoba in the case of *I. graellsii*, indicating that specimens from sample sites located close to each other or far away are very similar, as the distances between groups showed. These facts further strengthen our opinion to maintain specific status for the two studied taxa.

Given the morphological and ecological similarity between these species, it is likely that competition plays a crucial role in their partially exclusive distribution. Distribution maps published by several authors were suggestive of a non-overlapping distribution (e.g. Askew, 1988), but *I. elegans* seems more common in recent years. Several observations suggest that *I. elegans*, is spreading in NW Spain. In the 1980s, the population of Louro was sampled by Ocharán (1987) and it showed only *I. graellsii*, while in this research, only *I. elegans* specimens were found. One sample taken by us in 1995 had both species in similar frequency (and some interspecific matings and
intermediate individuals were found), but since 1998 we have found only *I. elegans*. Furthermore, the sympatric population of Foz included in this study (sampled in 1990) was revisited in 2001 and only *I. elegans* was found. During mark–recapture experiments in two *I. graellsii* populations, some specimens of *I. elegans* were found at a very low frequency (four out of 1600 (Cordero, 1989); five out of 1600, Cordero, unpubl. data), indicating that the latter species is dispersing in the area. To summarize, we found at least six localities that had both species simultaneously and that now have only *I. elegans*, but none in the opposite trend. We therefore suggest that the unidirectional mating that we have discovered is negatively affecting *I. graellsii*, whose females readily mate with the wrong male, and this might explain why the unidirectional mating that we have discovered is negatively affecting *I. graellsii*, whose females readily mate with the wrong male, and this might explain why

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