Single-Locus Complementary Sex Determination in Diadegma chrysostictos (Gmelin) (Hymenoptera: Ichneumonidae)

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Following the establishment of isofemale lines and subsequent inbreeding, the ichneumonid parasitoid wasp Diadegma chrysostictos (Gmelin) was shown by segregation of polymorphic alloenzyme loci to have single-locus complementary sex determination (sl-CSD). This and the biparental nature of diploid males was confirmed using two independent Mendelian recessive phenotypic markers. The existence of diploid males, sl-CSD, and the abrogation of diploid males following outbreeding was further confirmed by flow cytometry, a potentially general method that is independent of the maternal sex allocation or the need for genetic markers. Estimates of the number of sex alleles in several British populations demonstrated 17–19 alleles in Britain, with a decline toward the northerly limit of the parasitoid’s range, varying from 16 in the south of England to 4–5 in central Scotland, in broad agreement with the rate of attainment of a male-biased sex ratio when used to establish en masse laboratory cultures. These data represent the second confirmation of the existence of sl-CSD in the Ichneumonidae (and the first in the Campopleginae subfamily), lending further support to the notion that sl-CSD was the ancestral condition in the Aculeata/Ichneumonoidea clade (Cook 1993a; Periquet et al. 1993).

Sex determination in Hymenoptera is based on undefined forms of haplo-diploid arrhenotoky (e.g., Bull 1981; Poirie et al. 1992). Under this system of reproduction, unfertilized eggs develop as haploid males and fertilized ova as diploid females. The removal of recessive lethals via hemizygotic male production, coupled with the maternal ability to fertilize each egg or not (sex allocation), may have played an important role in the evolution of eusociality and the female-biased sex ratios often associated with extreme inbreeding (Hamilton 1967). In contrast, for some hymenopteran species, inbreeding results in a male-biased sex ratio and in the occurrence of diploid males. Neither the molecular basis of arrhenotoky nor that of diploid male production is known, but the presence of diploid males has been explained genetically by complementary sex determination (CSD), involving either a single codominant multiallelic locus (sl-CSD) (Whiting 1943) or multiple codominant multiallelic loci (ml-CSD) (Crozier 1971). Under sl-CSD, diploid males and females develop from fertilized ova homozygous and heterozygous at the sex locus, respectively, while haploid males arise from unfertilized eggs. In the similar ml-CSD, diploid males develop only from those individuals homozygous at all sex-determining loci. Diploid males are generally effectively sterile, compounding the sensitivity of the sex ratio to inbreeding through induction of female pseudovirginity. They have been reported in 33 hymenopteran species with all the available data supporting sl-CSD rather than ml-CSD (reviewed in Cook 1993b; Cook and Crozier 1995; Periquet et al. 1993; Southamer et al. 1992). The widespread occurrence of sl-CSD in every superfamily examined except the Chalcidoidea (e.g., Cook and Crozier 1995; Luck et al. 1993) has led to the suggestion that sl-CSD must have been the ancestral sex determination mechanism in the Hymenoptera (Bull 1981). However, very few species (from only 8 of the 18 superfamilies) have currently been tested for CSD. This scarcity of data, particularly for the supposed CSD-negative Chalcidoidea superfamily, precludes a meaningful analysis of the evolution of sex determination in Hymenoptera. Indeed, firm evidence confirming that diploid males are biparental, as predicted by CSD theory, rather than the result of mutations leading to uniparental diploid males (Skinner and Werren 1980) has only been confirmed in two species, the ichneumonid Diadromus pulchellus Wesmael (Periquet et...
al. 1993) and the braconid *Bracon hebetor* Say (Whiting 1943). Furthermore, the existence of diploid males has been established in only six species within the Ichneumonoidea despite its suggested ancestral ubiquity in this superfamily (Stouthamer et al. 1992; Periquet et al. 1993).

The ichneumonid *Diadegma chrysostictos* (Gmelin), a member of the Campoplegini tribe (Campopleginae subfamily), is a polyphagous solitary endoparasitoid of Pyralid (Lepidoptera) larvae and is endemic to Europe, Asia, Canada, and northern America. *D. chrysostictos* was identified as a species likely to show s-CSD since its sex ratio is female biased in the field, but becomes increasingly male biased in laboratory cultures leading to eventual culture extinction unless prevented by occasional outbreeding (Butcher 1999). In contrast, other members of the Campoplegini tribe, including the *Diadegma* genus, have not been recorded as displaying either a male bias or extinction in long-term laboratory cultures, while gregarious and thelytokous species are known to exist. Species from this tribe, therefore, represent interesting experimental models for the investigation of hymenopteran sex determination.

We present data derived from studies using allozyme and genetic markers consistent with the notion that *D. chrysostictos* possesses s-CSD. This is further supported by flow cytometric discrimination between haploids and diploids over successive generations, allowing determination of the ratio of diploid progeny that are male (DMR) in each generation, independent of the maternal sex allocation or the need for genetic markers and backcrosses. Taken together, these data provide the second demonstration of the validity of the s-CSD model in Ichneumonidae (the first for the Campopleginae subfamily) and illustrate the potential usefulness of flow cytometry in the study of CSD in larger Hymenoptera species.

**Materials and Methods**

**Biological Material**

British populations of *D. chrysostictos* were obtained from granaries at MAFF, Slough, in 1993–1995 (Slough); from fields and granaries around Aylesbury and Oxford (Oxfordshire); Nantwich and Knutsford (Cheshire); Leeds, Selby, and Otley (Yorkshire); Lanark to Glasgow (Lanarkshire), and sites in Stirlingshire, Fife, and Angus (central Scotland) during 1994–1996 (mean 110, range 95–136, individuals per collection per site); and used to establish en masse and isofemale line cultures on fourth instar larvae of *Plodia interpunctella* (Hubner) and *Ephestia kuehniella* Zeller, reared on a food medium consisting per 200 eggs of 300 g wheat middlings, 100 g whole-wheat flour, 100 g oatmeal, 30 g maize, 50 g dried baker's yeast, and 60 ml glycerol. All cultures were maintained at 26 ± 2°C, 40–60% relative humidity, and a 16:8 light:dark cycle.

**Experimental Cultures**

*D. chrysostictos* is highly polyphagous and frequently fails to avoid superparasitism (Butcher 1999; Fisher 1959), which can result in considerable mortality to developing parasitoid immatures. In addition, both parasitoid mortality and maternal sex allocation is markedly dependent on host size, density and species, foundress effects, and parasitoid age (Butcher 1999; Fisher 1959). To enable the observed secondary sex ratio to be an accurate reflection of the primary sex ratio, and not distorted by mortality, experimental conditions were optimized for minimal parasitoid/host developmental mortality including superparasitism, and a high diploid sex allocation to increase the sensitivity of DMR evaluation. This was achieved by using large fourth-instar *P. interpunctella* or *E. kuehniella* larvae as hosts, reared as above, and parasitized with one parasitoid per patch (except for en masse cultures), premated to a 1–2-day-old male, and allowed access to 50–70 fresh hosts a day (Butcher 1999). Under these conditions mortality was less than 6% (including superparasitism), with an average fecundity and diploid sex allocation of 56.8 ± 7.3% and 61.2% ± 4.3%, respectively.

**Alloenzyme Polymorphism Detection**

Individual imagos were homogenized into 35 μl of 10% (w/v) sucrose, microcentrifuged at 13,000 rpm for 8 min, and 5 μl of the clear S/N resolved by nondenaturing 5–8% polyacrylamide gel electrophoresis before developing and screening for the indicated enzymatic loci (Prakash et al. 1969; Loxdale et al. 1983).

**Development of Genetic Markers in *D. chrysostictos***

Freshly eclosed males were fed ad libitum with ethyl methyl sulphonate [0.38% (v/v) in 0.4% (w/v) glucose, 0.4% (w/v) fructose] for 5 days and mated with virgin females from the second day onward. Isofemale lines were established from all the daughters (F1), and maintained with screening of the F1→F2 progeny for stable phenotypic markers. Mendelian inheritance and dominance/recessiveness were confirmed by standard wild-type F1 backcross analysis. The two stable mutant phenotypes utilized in this study—rosy (red eye coloration) and curl (curled forewing edge between Rs2 and Cu1)—are both recessive and show independent Mendelian inheritance with no sex linkage (Butcher 1999).

**Morphometric Distinction Between Haploid and Diploid Males by Wing Hair Cell Density, Neuronal Cell DNA Flow Cytometry and Gametic Tissue Cytology**

Parasitoid forewing hair cell density was measured by light microscopy with both stage and eyepiece graticules by a horizontal (1.00 mm) and vertical transect (0.60 mm) across the DS and D2 cells (bordered by Rs + M, pterostigma, Rs + 2r, 2rm, 1mcu, and Cu1; and by 1mcu, 2mcu, and Cu1, respectively; Eady 1974).

The DNA content of single-cell brain neuronal suspensions was flow cytometrically analyzed using propidium iodide fluorescence. Briefly, parasitoid brains were dispersed to a single-cell suspension by hand homogenizing in Ringers with trypsin/chymotrypsin/versene (0.25 μg/ml each) and then aspirating through a 12-gauge needle. The single-cell suspension was centrifuged (300g maximum) over a Ficoll-hypaque cushion from which interface cells were harvested, washed, and then fixed and permeabilized (1 acetic acid:1 ethanol:8 Ringers) prior to RNAase A digestion (10 μg/ml in Ringers). Approximately 1000 cells that had been FSC/SSC gated to exclude polyplid connective tissue cells were collected and the DNA content analyzed using a Beckton Dickinson Facscan and the "Paint-a-gate" software program. Virgin females and their sons were used for each experimental condition.
were used as diploid and haploid standards, respectively.

Cytological evaluation of ploidy by gametic cell karyotypic analysis was carried out by the lactic acid spread method of Hoshiba et al. (1989), where n = 13 (acrocentric) and 2n = 26, with the same ploidy controls.

**Establishment of Isofemale Lines and Parental-Progeny Matings**

Isofemale lines were established by standard virgin female × son mating and subsequent single brother-sister pair matings, except that the founding virgin mother was stored with ad libitum access to fresh 50% (v/v) honey solution at 10°C prior to son mating because of the long ova-imago developmental time relative to female imago life span at 26°C. This protocol was also necessary for daughter-father backcrosses.

**Estimation of the Sex Allele Number in *D. chrysostictos***

For each geographical region the number of sex alleles in the population was estimated from (1) heterozygotic male frequencies at the polymorphic enzyme loci Adh, Est-1, Est-2, and Pgi (Owen and Packer 1994); (2) from the pooled equilibrium frequency of diploid males, detected by flow cytometry, over five generations after establishing en masse cultures with ≥100 wild-caught females and males (Periquet et al. 1993); and (3) from determination of the proportion of matched matings (those that produce diploid males) after establishment of en masse cultures and isofemale lines (Cook 1993a; Owen and Packer 1994).

**Results**

**Decrease in the Sex Ratio and Eventual Extinction in *D. chrysostictos* Cultures**

All the en masse *D. chrysostictos* cultures showed a marked decrease in the female secondary sex ratio (Figure 1A) and maternal fecundity (Figure 1B) over each generation, although the latter stabilized after 5–6 generations. Both trends could be reversed by outbreeding, providing moderate support for the operation of CSD. No increase in the average mortality was noted in each generation, and this data therefore reflects a true sex ratio and fecundity alteration rather than an increased differential mortality. The same trends were noted between and within cultures established over three consecutive years, and also when this parasitoid was cultured on three other natural host species (data not shown). In all three of the years sampled, the more northerly, and especially the Lanarkshire and central Scotland isolates, which are at or near the northernmost limit of *D. chrysostictos*’ range in the UK (Butcher 1999), appeared the most susceptible to inbreeding (Figure 1). Of note, however, is the extinction of all en masse cultures under these conditions, usually after 12–18 generations (Figure 1A), although a few cultures survived for up to 24 generations. This is not predicted by sl-CSD with two sex alleles, where even with the lowest average female fecundity noted (35 progeny) culture expansion would be expected as long as a (secondary) maternal sex allocation of more than 5% fertilized ova was maintained (Butcher 1997; Stouthamer et al. 1992).

**Diploid Male Detection and Establishment of sl-CSD by Allozyme Analysis**

The overall level of alloenzyme diversity observed in the U.K. populations of *D. chrysostictos* was low (H = 0.032 for 24 loci) with only seven polymorphic loci detected (Acp, Adh, Est-1, Est-2, Ldh, Pgi, and Pgm), each with a low level of heterozygosity (Butcher et al, in preparation). However, within any geographical isolate, these alleles appeared to be in Hardy-Weinberg equilibrium (chi-square test), and thus were acceptable for use in detecting heterozygotic diploids.

The Est-2, Adh, and Pgi loci were chosen to assay for heterozygotic (diploid) males, as these were the only loci which were both polymorphic in all the geographical isolates and unambiguously resolvable.
Females were singly mated with a male from the same culture, and two daughters (F₁) from each cross were backcrossed to their respective father. Parents were screened to identify crosses which involved a female heterozygous at any of the three chosen loci. In all crosses analyzed (Table 1), F₂ heterozygotic (diploid) males were observed, strongly supporting the operation of CSD. Although the observed allosex frequencies cannot be pooled for analysis due to individual maternal sex allocation differences, analysis of the progeny of each individual cross is compatible with sl-CSD in all cases, but incompatible with two-locus CSD (and thus ml-CSD) in 19 of 26 crosses. Note that the distribution of these alleles is not compatible with sex linkage, confirming the assumption of independence.

Genetic Confirmation of Biparental Diploid Males and sl-CSD in *D. chrysostictos*

Fifteen wild-type females (F₀) were crossed to *rosy* males and 15 to *curl* males (one male per female), and F₁ daughters from each cross were backcrossed to their respective father. From each of the F₂ progeny, *curl or rosy* F₂ daughters were mated to wild-type F₂ brothers, and the F₃ progeny sexed and scored for haploids (curl or rosy) and diploids (wild type). F₂ crosses that failed to yield any F₂ daughters (6/68 curl and 5/64 rosy) were excluded from the analysis, although this level of assumed pseudovirginity is within sl-CSD predictions.

In all *rosy* (59) and *curl* (62) crosses (F₂), analyzed, biparental diploid males were detected in the progeny (F₃) at a 44.6–49.5% DMR, in agreement with sl-CSD (50% DMR expected χ² = 0.0–0.37; all P > .50), but not ml-CSD (25% DMR expected for two loci, χ² = 6.8–17.2; all P < .01). More loci would further reduce the expected DMR. Analysis of the pooled DMR for all *rosy, curl*, and both crosses (2012 daughters, 1952 diploid males, and 3105 haploid males) is consistent with sl-CSD (χ² = 0.658, 0.297, and 0.908, respectively, all ns), but inconsistent with ml-CSD (χ² = 667, 735, and 1402, respectively; all P < .001) and therefore ml-CSD is excluded.

**Neither Wing Hair Cell Size nor Body Size Are Reliable Markers for Distinguishing Diploids from Haploids**

Diploid males have larger bodies (e.g., Periquet et al. 1993; Ross and Fletcher 1985) or wing hair cells (Grosch 1945) than their haploid counterparts. Potentially both these differences offer simple methods for haploid/diploid male discrimination and could facilitate detailed studies of the DMR over successive generations. Accordingly, 700 uniparental males, biparental males, and females from *rosy and curl* marked crosses were assayed for size (head to genitalia), forewing hair cell density, somatic cell DNA content, and gametic tissue ploidy by cytology, and compared to wild-type females and uniparental males (sons of virgin mothers) as diploid and haploid standards, respectively (Figure 2).

The adult body size and coloration was markedly dependent upon host species and rearing temperature, especially with smaller pyralid host species (Butcher 1999; Fisher 1959). Such variation invalidates this method for the analysis of field samples of *D. chrysostictos*. However, even when reared on the same host species at the same temperature, the near total overlapping size distribution precludes this method for the analysis of laboratory cultures. Thus although the mean size of diploid males (similar to that for females) was significantly larger than that for haploid males, at the individual level the size variation and resultant overlap was too great to allow a reliable distinction between haploid and diploid individuals (Figure 2A).

Although wing hair cell density appeared relatively insensitive to developmental temperature and host (data not shown), this method was also found to be unreliable for the analysis of field or laboratory-derived samples. Two different distributions were observed for diploid and haploid males, but the overlap in the size distribution accounted for some 28–32% of the males in the population sampled (Figure 2B).

In contrast, flow cytometric analysis of neuronal cell DNA content was independent of host species and developmental temperature (data not shown), and allowed unambiguous distinction between haploid and diploid individuals. Indeed the flow cytometric haploid/diploid discrimination was in absolute agreement with the genetic analysis (Figure 2C) and gamete tissue cytology (not shown) in all individuals assayed. However, this was not the case for all insect tissues tested, but only neuronal cells, as it was not possible to distinguish haploids from diploids uti-
Figure 2. Morphometric haploid-diploid discrimination in *D. chrysostictos*. The normalized (per 100) data for 700 genetically determined uniparental males, biparental males, biparental females, wild-type females, and haploid males reared from *E. kuehniella* and assayed for (A) body size, (B) wing hair cell density, and (C) brain neuronal DNA content as the mean relative fluorescent index (MFI). The average MFI ± SEM observed was genetically determined haploid males (65.6 ± 3.8), diploid males (122.8 ± 3.8), and females (122.2 ± 4.1), and wild-type haploid males (65.3 ± 4.6) and females (121.9 ± 3.5). Overlapping haploid/diploid body and wing hair cell size distributions were noted when reared on another three different host species and four different temperatures (data not shown).

**Flow Cytometric Confirmation of sl-CSD in *D. chrysostictos***

Flow cytometric analysis of the progeny of en masse cultures with two (established from the progeny of a single isofemale line) or more sex alleles confirmed the presence of diploid males at levels consistent with those predicted by sl-CSD (but not ml-CSD) under a maternal sex allocation of 60–65% in broad agreement with the observed sex allocation under these conditions (not shown).

To confirm sl-CSD independently of estimating the maternal sex allocation and the associated assumptions (Butcher 1999), strict isofemale lines were established for each of the *D. chrysostictos* isolates. *F*₀ daughters from each isofemale line were either sibmated to continue the subline or were outbred with males from a different geographical isolate, and the progeny DMR assayed (Tables 2 and 3).

Following son mating (*F*₀), diploid males were detected in the *F*₁ progeny of all isofemale lines with an individual DMR close to 50% as predicted by sl-CSD, but not ml-CSD (Table 2). Subsequent generations (*F*₂±*F*₆) also showed a DMR of near 50%, again in agreement with sl-CSD but not ml-CSD.

The abrogation of diploid males in the next generation following outbreeding, as predicted by CSD, was confirmed in all crosses (Table 3). Furthermore, reestablishment of isofemale lines from the diploid-male-free outbred lines (*F*₃ and *F*₄) resulted in an average progeny DMR near 50% in the next and subsequent isofemale generations, consistent with sl-CSD and not ml-CSD. Indeed all lines outbred with at least four other geographical isolates displayed an average DMR close to 50% in the first generation after isofemale inbreeding (not shown), consistent with sl-CSD rather than ml-CSD with all loci bar one fixed homozygotic. Finally, the segregation of individual outbred crosses with a DMR of 45–53% was also in close agreement with the predictions of sl-CSD and not ml-CSD.

**Sex Allele Numbers in *D. chrysostictos* at Different U.K. Locations**

Estimates for the U.K. as a whole revealed 17–19 sex alleles over the 3 years sampled, but showed a general trend of decreasing numbers of sex alleles in populations sampled toward the northern (more isolated) limit of this parasitoid’s range in the Unit-
ed Kingdom (Table 4). Thus the regional estimates ranged from 14 to 16 in south England (Slough not shown) and Oxfordshire through 9–11 in northern England (Cheshire and Yorkshire) and down to 5–6 and 4–5 in Lanarkshire and central Scotland, respectively. All three methods of estimation yielded essentially similar results.

**Discussion**

*D. chrysostictos* exhibited a decreased secondary sex ratio in en masse laboratory cultures, and this was enhanced under conditions of predominantly sibling mating and reduced by outbreeding. Furthermore, an inbreeding fecundity suppression was noted, suggesting that this species normally avoids repetitive inbreeding. Taken together, these observations made *D. chrysostictos* a good candidate for sl-CSD.

The data presented in this article strongly support the notion that *D. chrysostictos* is subject to sl-CSD, and confirm the biparental origin of the observed diploid males. In all experimental crosses evaluated (2158) in this study, diploid males were detected at levels predicted by the sl-CSD model, but too high for ml-CSD. One problem with the evaluation of CSD in laboratory cultures is the possibility of ml-CSD being mistaken for sl-CSD throughfixing of all but one sex locus as homozygotic over successive inbred generations. However, a near 50% DMR was noted in the F1 progeny of all fertile isofemale lines across all the geographical regions sampled over 3 years, and in the first generation of all isofemale lines established following repeated outbreeding of daughters with males from different geographical isolates. Similar DMRs were seen in the first generation of isofemale lines established immediately from field collected samples and after a single generation in the laboratory to allow outbreeding crosses between different collections. As it is most improbable that only one allele per locus was sampled over all geographical locations over 3 years for all the sex loci except one, then these data strongly support sl-CSD.

Our confirmation of sl-CSD in *D. chrysostictos* is consistent with the notion that sl-CSD was the ancestral condition in the Aculeata/Ichneumonoidea clade and perhaps within the Ichneumonoidea superfamily. However, this hypothesis requires further testing through studies on more species from across all tribes, sub- and superfamilies of the Hymenoptera. One major barrier to such investigations has been the difficulty in obtaining suitable genetic markers and the requirement for several generations of inbreeding for their characterization, which can lead to homozygotic fixation of sex loci, and erroneous interpretation of ml-CSD as sl-CSD (Cook 1993a,b). The need to circumvent these problems has led workers to exploit the physiological differences between haploids and diploids in body size, mass, or wing hair cell size (e.g., Grosch 1945; Ross and Fletcher 1985). However, in *D. chrysostictos* we found that neither body size nor wing hair cell density are reliable parameters for haploid/diploid discrimination. However, wing hair cell density in *D. chrysostictos*

<table>
<thead>
<tr>
<th>Isofemale generation</th>
<th>Isofemale lines established from <em>D. chrysostictos</em> wasps collected at the indicated sites</th>
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<tbody>
<tr>
<td></td>
<td>Oxonshire</td>
</tr>
<tr>
<td>F1</td>
<td>47.9 ± 2.4 (20)</td>
</tr>
<tr>
<td>F2</td>
<td>48.3 ± 1.6 (36)</td>
</tr>
<tr>
<td>F3</td>
<td>48.9 ± 1.0 (42)</td>
</tr>
<tr>
<td>F4</td>
<td>0.1 ns/90***</td>
</tr>
<tr>
<td>F5</td>
<td>47.9 ± 2.1 (29)</td>
</tr>
<tr>
<td>F6</td>
<td>51.1 ± 0.9 (25)</td>
</tr>
<tr>
<td>F7</td>
<td>0.4 ns/12***</td>
</tr>
<tr>
<td>x1</td>
<td>48.9 ± 0.5 (31)</td>
</tr>
<tr>
<td>x1</td>
<td>0.2 ns/2.72*</td>
</tr>
</tbody>
</table>

The average progeny DMR ± 1 SEM derived from the indicated number of female isofemale line crosses in parentheses carried out in 1995 and 1996. Infertile crosses (excluding the analysis) accounted for 2.1% of all F1 progeny, and 37.8% of all F2 progeny (F2 progeny, x1 analysis of the observed DMR are shown as (sl-CSD/ml-CSD) with the same key as in the legend to Table 1, with expected DMRs of 50% (all generations) for sl-CSD, and 25%, 30.5%, 34.2.5%, 39.6%, and 43.6% for ml-CSD (based on two, initially heterozygotic loci) over the F1 generation.
should allow selection of diploid or haploid males from a given laboratory population, for example, in testing diploid male sterility or mating strategies. In contrast, the flow cytometric analysis of neuronal cell DNA content, although destructive, unambiguously discriminated between haploid and diploid individuals without the need for genetic markers and backcrosses, and was independent of the parental genetics or maternal sex allocation. Whether neuronal tissues generally show low levels of polyplody in all hymenopteran species is not known, but flow cytometric haploid/diploid discrimination has been achieved in eight other ichneumonoidean parasitoids (data not shown). Thus this method, which is more reliable and reproducible than cytological analysis of imago gametic tissues, may prove to be broadly applicable to all medium-size or larger Hymenoptera, and should facilitate further research into hymenopteran sex determination and its relationship with mating strategies.

Given that under sl-CSD the number of sex alleles in a panmictic population influences the frequency of diploid males, and assuming reduced diploid male fertility or sterility, selection would be expected to favor an increase in the number of sex alleles within a population and inbreeding avoidance. Sex allele estimates for D. chrysostictos were 17–19 in the United Kingdom but ranged from 16 to 17 in southern England, down to 4–5 toward the extreme limit of this species range in Scotland. This high number of sex alleles at one locus is thus an expected result of selection to reduce the frequency of diploid males, and is in close agreement with estimates from the other sl-CSD species such as Apis mellifera, Solenopsis invicta, and Diadromus pulchellus, but higher than the nine alleles found in Bracon hebetor (reviewed in Periquet et al. 1993), although that for B. hebetor is likely to be a serious underestimation, at least within European populations (unpublished data). Although the actual numbers of sex alleles in these populations are probably higher than that estimated by our sampling efforts, the marked variation in the numbers of sex alleles between geographic populations is not likely to be an artifact of sampling, since similar numbers of parasitoids were sampled from each region, while the population size decreases northward. Thus we actually sampled a larger percentage of the field population in the northern regions, which displayed a lower frequency of sex alleles.

The absence of information on dispersal instances, immigration, and overwintering foundress numbers (and hence gene flow) restricts further analysis, but with this reservation in mind it is still tempting to speculate that the reduced sex allele levels noted at the northern extremes of D. chrysostictos’s U.K. range reflect the greater isolation of these populations. There is some support for this notion from the observed reduced genetic diversity (H and immigration (Nm), and increased inbreeding (Fis.values) in northern England, and especially Scottish, populations (unpublished data). With only 4–5 sex alleles, these semi-isolated northernmost populations would be expected to have been inbreeding-sensitive under sl-CSD and, with an estimated three to five overlapping generations a year (Butcher RDJ, personal observation) to have exhibited diploid males at frequencies of about 15–38% of all males, assuming an average maternal sex allocation of 50–70%. If this speculation is correct it raises the question as to why diploid male imagoes are neither detected at these sites, nor found in adults eclosing from field collected parasitized hosts (where differential mortality or dispersal cannot apply) throughout the year (Butcher et al., in preparation). A randomly mating population with 14–16, 10–12, and 4–5 sex alleles would be expected to have a sex allocation of 55–57%, 57–59%, and 70–73%, respectively, to remain stable (Cook 1993b). Although this is within the secondary sex allocation range observed in the field, in laboratory cultures the maternal sex allocation was found to be independent of either the geographical location or the estimated number of sex alleles in the population culture, but rather depended on the host species parasitized and the number of foraging females (unpublished data). This discrepancy could be explained if our estimated sex allele frequencies across the region do not represent the actual long-term “regional” levels. Alternatively, it is possible that avoidance of mating with sex allele-related males is occurring to overcome the fitness costs of inbreeding, or it could be that a more complicated sex allocation and sex determination system occurs than our experiments have suggested.

Finally, en masse laboratory D. chrysostictos cultures declined in secondary sex ratio to eventual extinction, as has been observed elsewhere (Fisher R, Marris G, Harvey I, and Reed D, and Davis A, all personal communications). Such an extinction is not predicted by sl-CSD, even with only two alleles, and allowing for observed inbreeding fecundity suppression. Although this discrepancy may be accounted for largely by the highly adaptive sex allocation of females in en masse cultures and the role of sterile diploid males under polyandry (Butcher et al., in preparation), this does not account for such a rapid extinction, and thus it cannot be excluded that additional sex ratio distorting factors are involved. The rate of decline and eventual culture extinction was not altered by a broad range of antibiotics or heat treatment (unpublished data), casting doubt on the hypothesis of bacterial-induced cytoplasmic incompatibility (Breeuwer et al. 1992; O’Neill et al. 1992), or son killer-type activity (Skinner 1985). It thus remains to be determined if D. chrysostictos is subject to other microbial or viral sex ratio distorters, or to self (nuclear) incompatibility, or other sex-distorting extrachromosomal elements akin to psr (Skinner 1985; Werren et al. 1987). The latter possibility could be addressed by a more thorough cytological examination of larval gametic metaphase spreads to distinguish the presence of any B chromosomes from acrocentric chromosomes.

Table 4. Estimated sex allele levels in D. chrysostictos from different localities in the United Kingdom

<table>
<thead>
<tr>
<th>Estimation method</th>
<th>Oxfordshire</th>
<th>Cheshire</th>
<th>Yorkshire</th>
<th>Lanarkshire</th>
<th>Central Scotland</th>
<th>Σ UK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allozyme</td>
<td>14 16 16 11 13 11 8 9 11 5 6 5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>18 18 19</td>
<td></td>
</tr>
<tr>
<td>En masse</td>
<td>17 16 17 10 11 11 9 10 10 6 7 6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>17 19 19</td>
<td></td>
</tr>
<tr>
<td>Isofemale</td>
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<td>4 5 4</td>
<td>18 17 18</td>
<td></td>
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</tr>
</tbody>
</table>

Population sex allele levels were estimated for D. chrysostictos from each geographical isolate from (1) the frequency of heterozygotic males at any of the investigated alloenzyme loci; (2) the equilibrium level of diploid males in en masse cultures; and (3) proportional analysis of matched matings in isofemale line crosses by progeny DMR analysis. Sex allele numbers are rounded to the nearest integer.
References


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