

A TEST FOR REPRODUCTIVE SEPARATION OF ALTERNATE GENERATIONS IN A BIENNIAL SPIDER, *ARANEUS DIADEMATUS* (ARANEAE, ARANEIDAE)

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ABSTRACT. In Denmark, two seemingly distinct size-classes, 3rd and 4th instar juveniles and reproductive adults, of *Araneus diadematus* are found during every breeding season in autumn, indicating a non-overlapping biennial life-cycle. We tested the hypothesis that alternate generations might experience a degree of reproductive isolation, using the distribution of nuclear (allozyme) and maternal (mtDNA) genetic markers. Individuals of a locality behaved as belonging to a random mating population, irrespective of size. No differences were found between any size-class pairs, within and between 2 yr, or among geographically distant samples. Processes that may lead to this result are discussed: the biennial development may be incomplete; or there may be migrational influx of genes from southern annual populations. There is no evidence for sexual differences in life-cycle length.

Keywords: Araneae, life history, biennial reproduction, mitochondrial DNA, allozymes

Several species of spiders at northern latitudes need an extended time to reach sexual maturity compared to their more southern conspecifics (Toft 1976 and references therein). The ecological background promoting such a life-cycle shift may result from lower temperature and shorter seasonal length (Roff 1992). Life-cycle shifts may also be viewed as a strategy to maximize fecundity by avoiding intraspecific competition (Polis 1984; Roff 1992). Most recorded life-cycle shifts in spiders involve species that extend their annual life-cycle in south and central Europe to a biennial life-cycle in northern Europe (Almquist 1969; Toft 1976). If the biennial development is perfect, cohorts that mature in even and uneven years will never mix. Dividing conspecifics into separately reproducing cohorts can foster sympatric race formation and, ultimately, speciation. In this manner, the 13-yr and 17-yr periodical cicadas have evolved into distinct evolutionary units (Archie et al. 1985; Marshall 2000).

The life-cycle of *Araneus diadematus* Clerck 1757 is generally described as annual (Bonnet 1930). However, in northern regions of Europe *A. diadematus* needs two years to

reach sexual maturity (Toft 1976). In Denmark, *A. diadematus* reaches maturity in July–August and mates through August and early September. The females lay a single egg sac in October. The egg sac overwinters, while the parent generation disappears before winter. Reproduction in spring has never been observed. In the breeding season two seemingly distinct size-classes can be observed: ca. 3rd to 4th instar juveniles (i.e., young of previous year's reproduction) and sexually mature animals (two years old). This pattern has consistently been observed during the past 30 years (Toft 1976; Toft pers. obs.). Analysis of size structure in the field produced no evidence for different developmental patterns in males and females (Toft 1976). It seems as if populations of *A. diadematus* have split into two non-overlapping cohorts.

The combination of a narrow breeding season and the repeated annual occurrence of two distinct size classes led us to speculate whether *A. diadematus* experiences some degree of reproductive isolation, i.e., whether size classes constitute two discontinuous cohorts. We tested reproductive separation assuming two hypotheses. First, reproductive separation can

be estimated as a departure from random mating between cohorts. This leads to the well-known Wahlund-effect (Wahlund 1928). Furthermore, given a 2-yr life-cycle, juveniles of a certain year should correlate genetically with adults of the following year, relative to adults of the same year. The same reasoning holds for adults of a certain year and juveniles of the next year. Second, a sex-specific developmental cycle is possible (cf. Levy 1970) though it seems to be rare, at least in temperate species (Schaefer 1987). If, for example, females mature in 2 years and males in 1 year, no differentiation of allozyme frequencies would be possible. However, if females' biennial development is complete, mtDNA might possibly have diverged. We tested this hypothesis using maternally inherited mitochondrial DNA.

METHODS

Juvenile (3rd and 4th instars) and adult spiders were collected at Mols, Denmark in the autumn of 1998 and 1999 (M98-J(juvenile), M98-A(dult), M99-J, M99-A) and from the 300 km distant island of Bornholm, near Hammern, in 1998 (H98-J, H98-A). Each size-class, at each location, was treated as a sample. All collections were done in the month of September, i.e., at a time when all the breeders of the year had matured. Bornholm is an isolated island in the Baltic Sea, approximately 30 km southeast of Sweden and 80 km north of Poland. The Bornholm samples were used to analyze the influence of geographic distance relative to genetic divergence between size groups, under the rationale that if two distinct reproductive groups exist, then correlations within size-groups should be independent of geographic distance.

All cohort related hypotheses were tested by applying biparental inherited markers (allozymes) and maternally inherited markers (mitochondrial DNA). Nine polymorphic loci, could be constantly scored in all samples: *Aat-2* (E.C. 2.6.1.1), *Fum* (E.C. 4.2.1.2), *Gpd* (E.C. 1.1.1.8), *Idh-1*, *-2* (E.C. 1.1.1.42), *Pgd* (E.C. 1.1.1.44), *Mpi* (E.C. 5.3.1.8), *Pgi* (E.C. 5.3.1.9), *Pgm* (E.C. 5.4.2.2). The locus *Adh* (E.C. 1.1.1.1) was also polymorphic but stained too weakly for juveniles to be analyzed, and was omitted from further analysis.

Electrophoretic analysis was done by cellulose acetate electrophoresis (Hebert & Bea-

ton 1993). A total of 121 spiders were investigated (see Table 1). Three buffer systems were used: Tris-Maleic acid pH = 7.0 (Richardson et al. 1986, adjusted with maleic acid to pH = 7.0 from pH = 7.8) for *Aat-2*, *Mpi*, *Pgd*. Tris-Citrate pH = 8.2 (Richardson et al. 1986) for *Gpd*, *Pgi*, *Idh*. Tris-Glycine pH = 8.5 (Hebert & Beaton 1993) for *Adh*, *Fum*, *Pgm*. All enzymes were run at 250V for 30 min.

Departure from random mating among juveniles and adults was tested as a departure from Hardy-Weinberg proportions for all spiders collected at Mols in the years 1998 and 1999, respectively, with the program GENEPOP (Raymond & Rousset 1995a). Differentiation among samples was quantified using the F_{ST} estimator of Weir & Cockerham (1984). F_{ST} is defined between 0 and 1, where $F_{ST} = 0$ signifies no variance among samples and $F_{ST} = 1$ signifies that all variance is distributed between samples. F_{ST} -analyses are based on allele frequencies summed over all loci. Divergence initially may proceed at a single locus, e.g., under selection, before genetic drift leads to divergent allele frequencies at other loci (e.g., McKechnie et al. 1975; Johannesson et al. 1995). Therefore, we tested for allele frequency homogeneity at each locus between all population pairs using exact tests (Raymond & Rousset 1995b) with GENEPOP. The relationship among juveniles and adults, and geographic locations was investigated applying maximum likelihood analysis with the program package PHYLIP (Felsenstein 1993). This algorithm assumes divergence by genetic drift only. Branch lengths are relative to divergence, and each is tested for significance.

Finally, we sequenced the mitochondrial DNA gene ND1 for 580 base pairs. ND1 has proven to be highly variable in other species of spider (Hedin 1997; Croucher 1998; Johannesen & Veith in press, Johannesen pers. obs.). Initially, two individuals from H98-J, and three individuals from all other samples were analysed, $n_{tot} = 17$. Amplification protocol and ND1 primers can be found in Hedin (1997). DNA fragments were sequenced in both directions. Sequences were aligned using the program Sequence Navigator and successively checked manually.

Table 1.—Allele frequencies at nine polymorphic loci for *A. diadematus*. For locality abbreviations please refer to the text.

		M98-J	M98-A	M99-J	M99-A	H98-J	H98-A
<i>Fum</i>	1	0.85	0.90	0.89	0.85	0.80	0.70
	2	0.15	0.10	0.11	0.15	0.20	0.30
<i>Aat-2</i>	1	0.96	0.96	0.92	1.00	1.00	1.00
	2	0.04	0.04	0.08	0	0	0
<i>Gpd</i>	1	0.21	0.27	0.13	0.25	0.15	0.07
	2	0.02	0	0	0	0	0
	3	0.77	0.73	0.87	0.75	0.85	0.93
<i>Idh-1</i>	1	0.81	0.81	0.92	0.90	0.80	0.91
	2	0.19	0.19	0.08	0.10	0.20	0.09
<i>Idh-2</i>	1	0.04	0.04	0	0	0.17	0
	2	0.96	0.96	1.00	1.00	0.83	1.00
<i>Pgd</i>	1	0	0	0.13	0.03	0	0.04
	2	0	0	0.05	0	0	0
	3	0.98	1.00	0.79	0.93	0.90	0.89
	4	0.02	0	0.03	0.05	0.10	0.07
<i>Pgi</i>	1	0.02	0.02	0.05	0.03	0	0.04
	2	0.62	0.40	0.53	0.53	0.40	0.50
	3	0.25	0.44	0.34	0.26	0.45	0.35
	4	0	0.04	0	0	0.10	0
	5	0.12	0.10	0.08	0.18	0.05	0.11
<i>Pgm</i>	1	0.02	0	0	0	0	0
	2	0.87	0.90	0.87	0.77	0.85	0.87
	3	0.12	0.10	0.13	0.22	0.10	0.13
	4	0	0	0	0	0.05	0
<i>Mpi</i>	1	0	0	0.04	0.06	0.09	0.02
	2	0.65	0.26	0.54	0.63	0.50	0.50
	3	0.31	0.52	0.32	0.31	0.41	0.35
	4	0.04	0.22	0.11	0	0	0.13
	<i>N</i>	26	24	19	20	10	22

RESULTS

Only a single mtDNA haplotype, thus no mtDNA differences among samples, was observed (Genbank number AY036084). We did not sequence further individuals after this result.

The allozyme allele frequencies are shown in Table 1. There were no allozyme-genetic correlations between alternate generations, nor were there deviations from Hardy-Weinberg proportions among generations within the same year at the same locality, $P > 0.50$. No allozyme differentiation was found among all samples, $F_{ST} = 0.018 \pm 0.013$, and no internal branches of the maximum likelihood phenogram were significantly greater than zero (tree not shown). No among-sample allele frequency differences were found in the loci *Pgi*, *Pgm*, *Idh-1*, *-2*, *Aat* and *Fum*. For the loci *Mpi*, *Pgd*, and *Gpd* allele number differences

were found for a single deviating sample, relative to all other samples.

Thus, neither hypothesis could be confirmed. There were no genetic differences between juveniles and adults of the same year, no genetic correlations between alternate generations, and no differences among females from different years. Furthermore, there were no genetic differences between geographically distant samples.

DISCUSSION

This study was inspired by the notion that the recurrent appearance in autumn of two size classes of *A. diadematus*, coupled to its narrow breeding season, could be related to reproductively separated cohorts. However, a lack of differentiation among samples was observed for nuclear genetic markers and no variation was observed in maternally inherited

mtDNA markers. No correlations between size classes or sexes were found. Thus, the genetic markers were not able to separate the size groups into two reproductive cohorts.

A lack of genetic correlation does not prove that *A. diadematus* size-classes are, to some extent, reproductively isolated. Lack of genetic correlations can be a consequence of large population sizes that cause genetic drift to have little power in separating cohorts. However, genetic homogeneity at nine allozyme loci suggests that cross-generation matings may not be uncommon. It is unlikely that too-small sample sizes resulted in a lack of significant correlations because alleles of the most polymorphic loci, where the sample error should be greatest, were all homogeneously distributed. It is more likely that the biennial life-cycle of *A. diadematus* is not complete, i.e., that a small fraction of the population (and probably both sexes) cross the generations by having annual or triannual life-cycles. Such mixed life-cycles are well known in several species of spiders (Toft 1976, 1983), but they are recognized easily only by standard analysis of size distributions if the developmental lines are numerically well represented. More extensive sampling or laboratory breeding studies may elucidate this possibility.

Uniform distributions of allozyme alleles from two locations 300 km apart indicated that gene flow may occur over large distances. High mobility by ballooning (Foelix 1996) is a possible reason for lack of differentiation both between the geographically distant samples (Ramirez & Haakonsen 1999) and between alternate cohorts. Furthermore, especially for mtDNA, lack of variation may be influenced by post-glacial colonization due to founder events. Colonization is not expected to influence the variation of nuclear genes as much because the effective population size of nuclear genes is four fold that of mtDNA. The level of allozyme polymorphism in *A. diadematus* is similar to that found in other spiders examined in northern Europe. These studies were able to separate local populations (Pedersen & Loeschcke 2001; Johannesen & Veith 2001) and even delimit micro-structures within populations (Johannesen et al. 1998; Schäfer et al. 2001).

In the face genetic homogeneity, caused either by gene flow or a recent life history split,

the recurrent occurrence of two size classes suggests strong selection for maintaining biennial life cycles. The phenomenon of divergence of biennial northern and southern annual populations remains an intriguing question. Immigration from central populations is thought to impede genetic adaptations of peripheral populations (Hoffmann & Blows 1994). Given a high dispersal potential, it is possible that immigrants from southern populations with an annual life-cycle can hinder separation of distinct cohorts, in that mobility among populations will break down any sign of intra-population cohort divergence. Therefore, the question arises about the extent of dispersal mobility of *A. diadematus*.

Reports on ballooning activity of *A. diadematus* are few and inconclusive. During analysis of seven year's catches by a 12.2 m high Rothamsted suction trap near Copenhagen, Denmark, Toft (pers. obs.) found four individuals, all 3rd or 4th instar juveniles. Three were captured in August–October of their first year, and one in June of the second year. Animals that are caught in this trap are assumed to be long-distance dispersers, because the catch is independent of the immediate surroundings of the trap due to its height. Though the number trapped is rather low, the data suggest that dispersal is predominantly in medium-sized instars, in which both autumn and spring dispersal is possible. Relatively, ballooning activity may be higher than the figures seem to indicate if related to the populations on the ground. *Araneus diadematus* is a widespread species, but it occurs only locally in high densities. It may thus be sufficiently active as a balloonist to explain the lack of geographical differentiation. The zone of transition between annual and biennial life-cycle of *A. diadematus* is unknown but may be only a few hundred kilometres south of Denmark. Even if it is unlikely that the distance may be bridged regularly by single individuals, accumulated dispersal over some generations may create the gene flow necessary to prevent genetic divergence of alternate biennial generations, even if there are no local "cross-overs" between alternate generations. In conclusion, we suggest that both intra- and inter-population gene flow may play a role in preventing intra-population differentiation.

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