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POSTOVULATORY FOLLICLES AS A MEASURE OF CLUTCH SIZE AND BROOD PARASITISM IN EUROPEAN STARLINGS[1]

E. DALE KENNEDY, PHILIP C. STOUFFER, AND HARRY W. POWER
Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855-1059

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Postovulatory follicles (POFs), the split, yellowish chambers remaining in a bird's ovary after ovulation, have been used as a morphological index of the number of eggs laid by individual females (Kabat et al. 1948; Davis 1958; Payne 1966, 1973, 1977). Generally, numbers of POFs have agreed with numbers of eggs laid (e.g., using histological techniques, Payne [1973] found agreement in numbers of POFs and eggs in the nest for 18 species). However, while examining the relationship of POFs to eggs in the nests of 32 European Starlings (*Sturnus vulgaris*), Davis (1958) found that matches of POFs and eggs in the nests occurred for only seven birds (22%), while 16 (50%) exhibited more POFs than eggs and nine (28%) had fewer POFs than eggs. As part of a study to examine whether starlings are determinate or indeterminate layers, and how this might relate to both intraspecific egg removal and brood parasitism, we replicated Davis' (1958) study.

METHODS

Fifty nest boxes were added to an existing starling nest-box trail in 1985 for the above study. (See Crossner [1977] for a description of the study site.) In 1986, starling nests were visited daily between 19 April and 12 May, and weekly thereafter until the end of May. Eggs were individually marked with indelible ink in the order that they appeared in each nest. Nests in which two new eggs appeared on the same day were noted as containing a parasite egg. Gaps in laying were also recorded, and birds from nests with laying gaps exceeding 1 day were excluded from analysis because there may have been a change in the resident female.

Fifty-two incubating female starlings were collected from nests 58–62 hr after the last egg was found in their nests; 33 birds were removed from early clutches (first egg laid in April), and 19 birds were removed from intermediate clutches (first egg laid in May). Each female was sacrificed within 2 hr of being collected, and her ovary was removed and fixed in Bouin's solution within 15 min of death. After 24 hr of fixation, each ovary was stored in 70% ethanol. Ovaries were examined at magnification 8–15× under a dissecting microscope. Starling POF counts were performed without knowledge of the number of eggs laid by each female in order to reduce potential bias in scoring, and each ovary was examined by the first two authors independently.

Ovaries from three birds were examined histologically to determine whether macroscopic POF counts were accurate or whether the oldest POFs, corresponding to the earliest eggs laid, had so regressed in size as to become undetectable. Ovaries were dehydrated, cleared, embedded in paraffin, serially sectioned at 8 μm, and stained with hematoxylin and eosin. Specimens were observed with a Zeiss stereozoom microscope at magnification 40–60×.

RESULTS AND DISCUSSION

Excluding known parasite eggs (those detected by egg censuses), only 28 of 52 birds (54%) had the same number of POFs as eggs found in the nest, and equal numbers (12 birds) had either more or fewer POFs than eggs (Table 1A). There are several reasons why larger counts of POFs than eggs may occur (Kabat et al. 1948, Davis 1958, Payne 1973). These include: (1) misidentification of atretic follicles (unovulated, often burst, degenerating follicles) as POFs; (2) ovulation of eggs into the body cavity rather than the oviduct; (3) laying of double-yolked eggs (each egg leaves behind two POFs in the ovary); (4) egg removal; and (5) laying of eggs outside the nest (in another bird's nest or on the ground). We observed atretic follicles in 15 of 52 ovaries, supporting Davis' (1942a) and Payne's (1966) conclusion that this is a common phenomenon. Atretic follicles contain unovulated oocytes and an amount of yolk that varies with their maturity (Davis 1942a). Such follicles are opaque, usually slitless, structures that are rounder and smoother than POFs (Payne 1966, 1973). We could not assess the frequency of factor 2 in our population; however, Payne (1973) observed only one ectopic yolk, which proved to be an entire follicle that had been severed from the ovary rather than an "ovulated" yolk, suggesting that ovulation into the body cavity is rare. We observed no double-yolked eggs among eggs removed from nests at the time of female collection, suggesting that factor 3 is a rare phenomenon in this population. Intraspecific egg removal and brood parasitism occur relatively frequently in this population (Stouffer et al. 1987, Romagnano 1987, respectively). Because it is unlikely that females with nests account for more than a small fraction of brood parasitism in our population (Romagnano 1987), most cases in which POFs exceeded the number of eggs are likely due to egg removal. Six of nine eggs found on the ground beneath study nests were marked, indicating that these

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TABLE 1. Number of nests with greater, equal, or fewer macroscopic postovulatory follicles (POFs) than eggs for different clutch sizes. A. True clutch size (excludes parasite eggs detected by daily censuses). B. Gross clutch size (includes parasite eggs detected by daily censuses).

	Eggs in nest	Number of nests	Relationship of POFs to eggs in nest		
			POFs > eggs (%)	POFs = eggs (%)	POFs < eggs (%)
A.	3	3	2 (67)	1 (33)	0 (0)
	4	17	4 (24)	9 (53)	4 (24)
	5	26	6 (23)	15 (58)	5 (19)
	6	6	0 (0)	3 (50)	3 (50)
Total		52	12 (23)	28 (54)	12 (23)
B.	3	3	2 (67)	1 (33)	0 (0)
	4	16	4 (25)	8 (50)	4 (25)
	5	24	6 (25)	13 (54)	5 (21)
	6	7	0 (0)	2 (29)	5 (71)
	7	2	0 (0)	0 (0)	2 (100)
Total		52	12 (23)	24 (46)	16[a] (31)

[a] Six cases of parasitism were detected by censusing; in all six nests, numbers of POFs were less than the numbers of eggs in the nest. In two of these nests, POF counts indicated a second parasite egg.

eggs had not been laid on the ground; all three unmarked eggs were broken, suggesting that they also had been removed from nests. In addition, marked eggs disappeared from six other nests.

These five factors may have been more important in the population studied by Davis (1958) because his sample, in contrast to ours, contained a significantly greater proportion of birds in which the number of POFs exceeded the number of eggs in the nest (16 of 32 vs. 12 of 52; $G = 6.392$, df = 1, $P < 0.025$; to be consistent with Davis' data, we used our gross clutch size, shown in Table 1B). Our boxes were in a more open habitat than those studied by Davis, and may have been occupied by more dominant starlings less likely to act as intraspecific brood parasites. Competition was keen for our boxes, and box use suggested a surplus of potential breeders. Forty-six of 50 boxes contained an early nest, and 31 of 33 early nests from which females were collected contained an egg from another breeding female within 2–26 days ($\bar{x} = 9.8$ days, SD = 5.4 days) of removal of the first female.

Two factors may result in fewer numbers of POFs than eggs in the nest: atrophy of POFs prior to collection and intraspecific brood parasitism (Davis 1942b, 1958; Payne 1966, 1973, 1977). Payne (pers. comm.) found a close correlation between macroscopic and microscopic counts of POFs in all of the species he studied. Moreover, he found that POFs in Tricolored Blackbirds (*Agelaius tricolor*) and Red-winged Blackbirds (*A. phoeniceus*) remained recognizable as macroscopic structures for at least 1 week after ovulation, and that POFs in blackbirds and viduine finches (*Vidua*) were recognizable histologically in serial sections for up to 14 days after ovulation (Payne 1966, 1977). Our starling ovaries were collected within a maximum of 7.5 days (for clutches of six) after the laying of the first egg. Our microscopic examination of three ovaries showed that the smallest POF dimension was 0.66 mm while the largest was 2.26 mm. Using the smaller dimension as a guide, we reexamined the other 49 ovaries for small POFs that we may initially have overlooked, and our results are based on this reexamination (Table 1). Intraspecific brood parasitism has been reported for many birds (Yom-Tov 1980), including starlings elsewhere (Yom-Tov et al. 1974) and in our population (Romagnano 1987). Our results agree with the finding that most brood parasitism in this population occurs in early clutches (Romagnano 1987); all six cases of brood parasitism detected by daily egg censuses alone, and eight of 12 cases in which number of POFs was less than true clutch size occurred in early nests.

The percentage of nests in which the number of eggs exceeded the number of POFs was similar in our study (31%, Table 1B) and in Davis' (1958) study (28%). In both studies the percentage of nests in which the number of eggs exceeded the number of POFs increased significantly with clutch size (this study, $r = 0.951$, df = 3, $P < 0.05$; from Davis [1958], $r = 0.899$, df = 5, $P < 0.01$). Because these values may represent estimates of the rate of intraspecific brood parasitism, the results may be interpreted as suggesting an increase in brood parasitism with an increase in gross clutch size. Other studies on our population also show this trend, and find a comparably high percentage of brood parasitism in clutches of six and seven eggs (Romagnano 1987; Power et al., unpubl. data).

We found that the rate of intraspecific brood parasitism in early broods revealed by POFs (12 of 33 nests, 36%) was higher than that revealed by daily egg censuses and electrophoresis (21%, Romagnano 1987) in the same population. This is not surprising given the conservatism of electrophoresis in our population (Romagnano 1987). We feel that use of POFs provides a less conservative and more accurate rate of intraspecific brood parasitism. However, POF counts are still conservative; a brood parasite may remove a host egg at the same time she adds an egg to a nest, as suggested by observations of Lombardo et al. (in press), and thus conceal instances of both intraspecific brood parasitism and egg removal. Our results reinforce those of Romagnano (1987) and Lombardo et al. (in press), showing that reproductive success in starlings is diminished by both intraspecific brood parasitism and egg removal. Therefore behaviors that prevent or reduce this loss of fitness should be favored.

Macroscopic examination of ovaries is a direct and inexpensive index of true clutch size, making it potentially useful in studies of egg removal and brood parasitism. However, its application is limited by the uncertainty that each POF in fact represents a single egg laid in a nest (i.e., eggs may be double-yolked or laid on the ground), the possibility of confusing atretic follicles with POFs, possible atrophy of early POFs in birds with large clutches, and the necessity of sacrificing birds as soon as possible after clutch completion, precluding subsequent observations and demographic measurements. Although the extent of the first limitation is unknown, it is probably not common in most wild birds, as double-yolked eggs are rare, and we found no intact unmarked eggs on the ground. The second and third factors can be minimized by systematic collection of females within 1–2 days of clutch completion.

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REGIONWIDE POLYGYNY IN WILLOW FLYCATCHERS[1]

JAMES A. SEDGWICK AND FRITZ L. KNOPF
U.S. Fish and Wildlife Service, National Ecology Research Center, 1300 Blue Spruce Drive, Fort Collins, CO 80524-2098

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Most species of North American flycatchers (Tyrannidae) are believed to be normally monogamous (Skutch 1960, Verner and Willson 1969). Some instances of bigamy are known for the Eastern Phoebe (*Sayornis phoebe*; Sherman 1952), Eastern Wood-Pewee (*Contopus virens*; W. J. Smith, cited in Eckhardt 1976), Western Wood-Pewee (*C. sordidulus*; Eckhardt 1976), and Acadian Flycatcher (*Empidonax virescens*; Mumford 1964). Recently, local incidences of polygyny have also been reported for the Least (*E. minimus*; Briskie and Sealy 1987) and Willow (*E. traillii*; Prescott 1986) flycatchers. Here, we present details on two additional instances of polygyny in Willow Flycatchers in different regions of North America, including information on the behavior and nesting ecology of polygynous trios.

We observed instances of polygyny in Willow Flycatchers in 1987 incidental to other studies on two national wildlife refuges in the western United States: Arapaho National Wildlife Refuge (ANWR) in north-central Colorado, and Malheur National Wildlife Refuge (MNWR) in southeastern Oregon. ANWR is located in a high-elevation (2,500 m), intermountain glacial basin. Willow Flycatchers in this area occur along the floodplain of the Illinois River, wherever there are extensive stands of shrub willows (*Salix* spp.). The shrub willow community at ANWR is dominated by four species of willows—*S. geyeriana, S. monticola, S. caudata,* and *S. planifolia* (Cannon and Knopf 1984). MNWR is located at the northern edge of the Great Basin province (elevation = 1,280 m). Flycatchers at MNWR occur along the Blitzen River, also in stands of shrub willows. *Salix exigua* and *S. lutea* dominate the willow community at MNWR.

We observed apparent polygyny at MNWR on 11 June. At that time most flycatchers were paired and in the early stages of nest construction. Birds presumed to be females were actively carrying nesting material, and birds presumed to be males were alternately accompanying females to and from nests and singing at

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