Spotless starlings (*Sturnus unicolor*) lay immaculate blue-greenish eggs. However, some days after incubation the eggs start to show fine red-brownish spots, becoming denser as the incubation progresses. It has been suggested that these spots could originate from the nest material or from some parasite living therein (Feare and Constantine, 1980).

The carnid fly *Carnus hemapterus* is a 2 mm-long fly that feeds upon the blood of nestlings of many bird species (Capelle and Whitworth, 1973; Fitzner and Woodley, 1983; Grimaldi, 1997; Roulin, 1999). It has been reported to be highly prevalent and abundant on colonially breeding starlings (*Sturnus vulgaris*), being the dominant blood-sucking ectoparasite of nestlings in this species (Liker et al., 2001).

The present study evaluates whether egg spots are related to infestation by *C. hemapterus* in the spotless starling. Correlative data between number of egg spots and number of *C. hemapterus* in the nest were recorded. Also, an experimental manipulation of the ectoparasite load was performed, and the number of egg spots was compared depending on whether nests had been sprayed with an avian insecticide (experimental group) or with water (control group).

The study was carried out in a colony of spotless starlings in Soto del Real, Madrid, during the breeding season of 2006. When laying started, nestboxes were visited daily and new eggs found were numbered with an non-toxic indelible marker. On day 4 of incubation the number of spots was counted in an area of 1 cm$^2$ on the lateral broad pole of each egg in the clutch. In every nest 1 or 2 eggs were cleaned by removing the spots with cotton damped in water, and a similar number of eggs were left as they were found. Also on day 4 of incubation, the number of *C. hemapterus* was counted by turning over the nest and shaking it over a white sheet of paper, where ectoparasites were counted. Bird mites (*Ornithonyssus bursa*, (Arachnida: Acari: Macronyssidae)) were found in 5 nests out of 15, however they showed no relation with the presence of spots ($F_{1, 13} = 0.24, P = 0.64$), and are not discussed further. Nests were randomly assigned to experimental or control groups. There was no difference in the number of *C. hemapterus* found in the nest before manipulation (Control nests = 16.64 ± 12.62, $n = 7$;
Experimental nests = 9.58 ± 6.24, \( n = 8 \); \( F_{1,13} = 1.64, P = 0.22 \). Nests were taken out of the box and sprayed with either an avian insecticide (based on 8% tetramethrine) or with water. We allowed a period of 5 minutes to let the substance dry before returning the eggs to the box. At day 8 of incubation (4 days after the experiment was performed) the same procedure was used for counting egg spots and *C. hemapterus*. Data were analysed using mixed models in STATISTICA 6, declaring nest as a random factor. Residuals of all models were normally distributed.

Results show that the number of egg spots was positively correlated with the number of *C. hemapterus* in the nest at 4 days of incubation (4 days after the experiment was performed): experimental nests had a significantly lower ectoparasite load than control nests (\( F_{1,45} = 25.23, P < 0.001 \); Fig 2). As expected, the number of egg spots was different between treatments: experimental nests presented significantly fewer spots than control nests (\( F_{1,9,8} = 100.01, P < 0.001 \); Fig 3).

To sum up, this study provides evidence that the spots on starling eggs are caused by the activity of carnid flies (*C. hemapterus*) living inside nest material. Previous studies show that the population of adult flies in nests increases from the hatching stage to the half of the rearing period and declines rapidly after feathers start to grow (Dawson, 1997; Liker *et al.*, 2001; Roulin *et al.*, 2003; Valera *et al.*, 2003). Therefore, it is interesting to note that we were able to find flies in the nest just a few days before incubation started. Until now,
there is no previous evidence that this fly infests nests before egg hatching.

Although there is no detailed knowledge about how these spots are formed, some anecdotal observations suggest that they are actually fly droppings and not blood marks from wounds in the brood patch of the female (cfr. Feare, 1984). This idea is supported by the fact that they are mainly distributed in the poles of the egg (a zone which has less contact with the brood patch and more contact with nest material). Regardless of the nature of the spots, the data presented here shows that egg spots are good indicators of ectoparasite load by C. hemapterus. It has been suggested (Moreno and Osorno 2003) that male birds can use egg colour to assess female quality and adjust parental investment accordingly. On the same line, and to the extent that males inspect eggs after incubation, our data show that in the spotless starling, males could potentially use egg spots as an indicator of brood parasite load.

Resumen.—Se ha sugerido que los puntos marrones que aparecen en los huevos de estornino negro una vez comenzada la incubación podrían relacionarse con la abundancia de ectoparásitos del nido. Para poner a prueba esta hipótesis se manipuló la carga ectoparasita de los nidos rociándolos con insecticida (grupo experimental) o con agua (grupo control) y se comparó el número de puntos entre ambos grupos. Se encontró una relación positiva entre el número de moscas (Carnus hemapterus) en el nido y el número medio de puntos en los huevos. Los nidos experimentales presentaron un menor número de puntos que los controles. Nuestro estudio demuestra que los puntos de los huevos de estornino negro son producidos por la presencia la mosca parasita C. hemapterus.

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