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Valor adaptativo de la incorporación de materiales urbanos en la construcción del nido; posibles ventajas de la plasticidad conductual

TESIS

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ÍNDICE

I. RESUMEN

Los ambientes urbanos, comúnmente, presentan condiciones nuevas y dinámicas a los organismos que habitan en ellos. En algunos casos, las especies pueden sobreponerse y obtener ventaja de las condiciones urbanas, llegando a ser habitantes exitosos de las ciudades. Un aspecto al que se enfrentan muchos organismos en las ciudades es la basura que, a veces, simula otros recursos naturales, por lo que puede afectar a la fauna silvestre. Las aves han sido objeto de estudio en muchos trabajos de ecología urbana, ya que muchas especies han colonizado exitosamente las ciudades a veces mediante ajustes de su conducta o historias de vida. Sabemos que diferentes aves de la Ciudad de México utilizan distintos materiales sintéticos o industriales, incluidas colillas de cigarro. Este material es parte de la estructura interna de los nidos, por lo que está en contacto con huevos, pollos y padres durante toda la época reproductiva. Muchos trabajos han mostrado que diversas sustancias presentes en el tabaco, y que se acumulan en los filtros al ser fumados los cigarros son tóxicas. En nidos de gorrión inglés y de pinzón mexicano los filtros están asociados negativamente a la cantidad de ectoparásitos (ácaros), por lo que usarlos podría ser benéfico para estas especies. Sin embargo, en este trabajo exploramos otras posibles consecuencias de agregar este material. Examinamos estas consecuencias durante el crecimiento de las crías y en los adultos que manipulan las colillas. Finalmente, a través de experimentación evaluamos la idea de que las aves responden activamente a la carga ectoparasitaria, ajustando el uso de colillas de cigarro en sus nidos. Encontramos que pollos y adultos muestran daño genotóxico asociado a la cantidad de filtros de cigarro en los nidos. Además, las aves agregan filtros de cigarro cuando aumenta la carga

ectoparasitaria, lo que también parece depender en parte de la experiencia previa. La prontitud con la cual estas especies incorporan en sus nidos este repelente de ectoparásitos, de origen antropogénico, tanto como la aparente capacidad para enfrentar sus efectos tóxicos, podrían contribuir a explicar por qué estas especies son tan exitosas en sitios urbanos. A la luz de nuestros resultados se hace evidente la necesidad de explorar las posibles consecuencias tanto para pollos como para los adultos, de la incorporación de materiales antropogénicos, en sustitución o además de los usados naturalmente en la construcción de nidos, al menos con tanto cuidado como se ha estudiado el efecto de los sitios de anidación, o las respuestas a contaminación acústica por aves urbanas.

Abstract

Urban environments present new and dynamic conditions to the organisms that inhabit them. In some cases, species can take advantage of urban conditions and become successful colonizers. Many organisms have rubbish in anthropogenic environments, which can simulate other resources and affect wildlife. Some bird species have successfully colonized cities by adjusting their behavior or life histories. Different birds of Mexico City use synthetic materials, such as cigarette butts, which form part of the internal structure of their nests. This material is in contact with eggs, chickens and parents during the breeding season. Several studies have shown that the substances present in cigarette and cigarette butts are toxic. In nests of house sparrows and house finches, butts are negatively associated with the amount of ectoparasites, so using them could be

beneficial. However, in this paper we explore other possible consequences of adding this material. We examined consequences for chick growth and for adults handling cigarette butts. Finally, we evaluated if birds respond actively to the ectoparasitic load by adjusting the use of butts in their nests. We found that chickens and adults show genotoxic damage associated with the number of butts in nests. In addition, birds add butts when the load of ectoparasites increases, which also seems to depend on previous experience. The readiness with which these species incorporate in their nests this anthropogenic repellent, as well as the apparent capacity to face its toxic effects, could contribute to explain why these species are so successful in urban sites. This study makes evident the need to explore the possible consequences of the use of anthropogenic resources by animals in cities.

II. INTRODUCCIÓN GENERAL

1. Los ambientes urbanos y las aves

Con la expansión de las ciudades, los humanos cambiamos nuestras condiciones de vida y la de otros animales y plantas, ya que modificamos el ambiente con el que muchas especies han interactuado a lo largo de su historia evolutiva. Estos nuevos ambientes introducen retos para muchas especies, debido a que las condiciones son muy diferentes de las que se encuentran o se encontraban en ambientes no perturbados (Vuorisalo et al. 2003, Blair 2004). Como consecuencia de las modificaciones al hábitat, algunas especies son excluidas, pero algunas se ajustan exitosamente a las nuevas condiciones (Ditchkoff et al. 2006). Esto sucede porque el cambio modifica las condiciones naturales que experimentan los organismos. Ello inicia un proceso de ajustes, frecuentemente conductuales. Así en las ciudades algunas especies tienen la oportunidad de tener ciclos reproductivos extendidos, recursos alimenticios abundantes, menor diversidad de depredadores y patógenos, sitios y materiales de anidación novedosos, etc. (Jokimaki and Huhta 2000, Ditchkoff et al. 2006). Es por lo anterior que muchos biólogos estamos interesados en estudiar y entender las consecuencias de estas modificaciones, en nuevos ambientes como las ciudades, en la biología de especies de animales y plantas (Marzluff 2001, Marzluff et al. 2001).

Debido a que las ciudades cambian en función de avances demográficos, industriales y tecnológicos, la plasticidad de caracteres morfológicos, fisiológicos y la innovación y plasticidad conductual pueden contribuir en las primeras etapas de la colonización de las ciudades (Gross et al. 2010). Estas características permiten la

explotación de nuevos recursos y ayudan a que los animales ajusten o modifiquen la forma en la que interactúan con el ambiente nuevo (incluso cuando no es un ambiente urbano; Piaget 1978, Snell-Rood 2013, Sol et al. 2013). Algunas especies, inicialmente, son capaces de explotar recursos que provee la ciudad además de los que se encuentran naturalmente (Mckinney 2002). Frente a la escasez de recursos, las especies potencialmente colonizadoras pueden reemplazarlos por otros de manera más eficiente que las más estereotipadas. Eventualmente, las poblaciones de éstas aumentarán, compitiendo exitosamente con las especies menos prontas a usar los recursos que ofrecen las ciudades (Beissinger and Osborne 1982, Blair 2001, Martin II and Fitzgerald 2005, Mckinney 2006).

El caso particular de las aves es muy interesante, ya que muchas especies han colonizado exitosamente las zonas urbanas, posiblemente por la forma en la que se mueven entre sitios (mediante el vuelo), su tamaño, su versatilidad alimenticia y su tolerancia a la presencia de humanos (Marzluff et al. 2001, Antczak et al. 2010). Por ejemplo, algunas especies aprovechan alimentos humanos, abundantes en las ciudades, como una alternativa en su dieta (Galbraith et al. 2015, Scott et al. 2015). Además, se han estudiado casos de ajustes de atributos del canto como la frecuencia mínima (Slabbekoorn and Peet 2003, Burhans and Thompson 2006, Bermúdez-Cuamatzin et al. 2009, Nemeth and Brumm 2009), la duración (Slabbekoorn and Den Boer-Visser 2006) y el momento del día en el que cantan (Gil et al. 2014). Es por esto que las aves son un sistema muy bueno para responder preguntas sobre ajustes a las ciudades y sus consecuencias (Ver apéndice para más información).

2. Construcción del nido

El grupo de las aves se distingue porque todas las especies son ovíparas. Debido a esto la construcción del nido es una parte muy importante de su reproducción (con algunas excepciones; Collias 1964). Se ha sugerido que la incubación parental de los huevos optimiza el desarrollo embrionario y puede haber favorecido la evolución del cuidado biparental al forzar a uno de los padres a efectuar tareas complementarias que el que incuba no puede realizar. Esta división del trabajo habría promovido la supervivencia de los pollos (Wesolowski 1994), al tiempo que la estructura del nido habría evolucionado al facilitar el cuidado parental (Collias 1964, Roff et al. 2005, Mainwaring et al. 2014, Macías Garcia et al. 2016).

Las aves se distribuyen prácticamente en todo el planeta, excepto en las zonas más frías de los casquetes polares. Los nidos suelen variar de un ambiente a otro, incluso entre poblaciones de una misma especie en función de los diferentes tipos de sustratos y de los materiales disponibles (Mennerat et al. 2009a, Álvarez et al. 2013). En el caso de la colonización de nuevos ambientes, las aves pueden explotar diferentes tipos de recursos, para construir y ubicar sus nidos. En las ciudades, la basura es una de las fuentes de recursos de origen humano que pueden tener disponible (por ejemplo para material de nido; Yeh et al. 2007). Naturalmente, la elección de sitios y materiales de anidación contribuiría a la seguridad de las crías, la regulación de la temperatura y la humedad. El uso de los materiales para la anidación estaría en función de su disponibilidad en las inmediaciones de los sitios de anidación o de las fuentes de alimento (Collias and Collias 1984, Macías Garcia et al. 2016). Por lo tanto, en los procesos de colonización de nuevos

ambientes la plasticidad e innovación de la construcción del nido podrían promover mayor supervivencia y mayor éxito reproductivo (Duckworth 2009).

2.1 Materiales del nido

La habilidad de las aves para elegir materiales correctos para la estructura del nido es fundamental para el éxito de la nidada (Bailey et al. 2014). Esta conducta incorpora toma de decisiones que dependen de la experiencia, el ambiente y la especie de la que se trate (Muth et al. 2013, Collias and Collias 2014), ya que determinan sus propiedades físicas (Nilsson 1984, Healy et al. 2008, Bailey et al. 2014, Muth and Healy 2014) y algunas veces químicas (Clark and Mason 1988, Gwinner et al. 2000).

El ensamble de materiales del nido es típicamente muy parecido a nivel de género, aunque también hay variación significativa entre individuos (Collias 1997, Hansell 2000). Esta flexibilidad puede ser adaptativa, cuando hay variación de materiales entre ambientes, y puede ser alcanzada a través del aprendizaje (Mennerat et al. 2009a). Bailey et al. (2014) evaluaron las hipótesis de que la elección de materiales depende de 1) la experiencia, 2) el éxito reproductivo previo o 3) la experiencia temprana, en el pinzón cebra (*Taeniopygia guttata*). Encontraron que lo más importante para estas aves fue la experiencia previa, ya que las aves eligieron los materiales que, en subsecuentes intentos de construcción, mejoraron la estructura del nido. Además en diversos estudios se ha encontrado que la experiencia mejora la elección de los materiales y los sitios correctos para construir el nido, resultando en un mayor éxito de la nidada (Muth and Healy 2011, Muth et al. 2013).

3. Efectos particulares de los materiales del nido

La mayoría de los materiales que las aves utilizan para construir sus nidos cumplen funciones de soporte y estructura, además de proporcionar condiciones térmicas y de humedad apropiadas. Adicionalmente, algunas especies incluyen elementos como materiales coloridos, hojas verdes y/o aromáticas (Pearce et al. 1997, Gwinner and Berger 2008, Mennerat et al. 2009a). Estos materiales pueden influir en las propiedades del nido (Brouwer and Komdeur 2004, Veiga et al. 2006, Moreno et al. 2010, Peralta-Sanchez et al. 2010).

Muchos investigadores han reportado casos de uso de materiales vegetales verdes. Se ha encontrado que esta conducta es importante, ya que, puesto que muchos linajes de plantas han evolucionado adaptaciones para combatir la herbivoría a través de sustancias volátiles (Wink 1988), algunas de las plantas que las aves eligen pueden tener propiedades repelentes (Clark and Mason 1988, Mennerat et al. 2009b). La supervivencia de los pollos y la salud de los padres frecuentemente se ven afectadas por los ectoparásitos malófagos y hematófagos (que además son vectores de endoparásitos) en los nidos (Lehmann 1993, Weddle 2000, Fitze et al. 2004, Cantarero et al. 2013, López-Arrabé et al. 2015). Uno de los ejemplos mejor estudiados del uso de materiales que asisten en el control de la cantidad de ectoparásitos es el de los estorninos europeos (*Sturnus vulgaris*). Clark y Mason (1987) encontraron que los estorninos europeos agregan hojas de distintas plantas en sus nidos, y que estas plantas son agregadas mientras están frescas, siendo reemplazadas constantemente. Con una cantidad muy baja de compuestos tóxicos en las plantas, los ectoparásitos no mudan o retrasan su reproducción, lo que provoca una

disminución de sus poblaciones. Uno de los aspectos más interesantes es que los estorninos prefieren ciertas especies de plantas, cuya función repelente es mayor. Ahora sabemos, en este mismo sistema y en otros más, que ciertos materiales en los nidos pueden tener efectos directos sobre la salud de los pollos y los padres (Clark and Mason 1988, Gwinner et al. 2000, Petit et al. 2002, Mennerat et al. 2009b, Peralta-Sanchez et al. 2010, Polo et al. 2010).

Los materiales, que han sido estudiados y reportados como repelente de ectoparásitos, son exclusivamente de origen vegetal (Dubiec et al. 2013). Sin embargo en las ciudades, la diversidad de plantas es muy inferior o diferente a la que se encontraría naturalmente o en el campo (Muratet et al. 2008). Su permanencia en estos ambientes alterados depende más de los beneficios (estéticos o ambientales) que obtienen los humanos por su presencia, que de su capacidad para enfrentar herbívoros (cuyo control recae en los humanos). Estas condiciones probablemente desfavorecen la presencia en las ciudades de aquellas plantas que podrían ser utilizadas como repelentes de ectoparásitos del nido en zonas urbanas.

3.1 El curioso caso de los filtros de cigarro

Recientemente encontramos que en un ambiente urbano, aves como el gorrión inglés (*Passer domesticus*) y el pinzón mexicano (*Carpodacus mexicanus*) utilizan filtros de cigarro en la estructura de sus nidos. Con base en los estudios sobre la utilización de plantas repelentes y a que los cigarros contienen tabaco (*Nicotiana tabacum*; reconocida por sus propiedades como repelente de artrópodos; Pascual-Villalobos 1998, Kaplan et al. 2008), encontramos que este material ayuda a las aves a reducir significativamente la abundancia de ectoparásitos (Suárez-Rodríguez et al. 2013). Sin embargo, a diferencia de las plantas, el cigarro no es un material completamente natural. El cigarro contiene otras sustancias sintéticas que son peligrosas para los humanos y otros organismos (Sopori 2002, Witschi et al. 2002, DeMarini 2004). Además, los filtros usados pueden retener del 25 al 30% de las sustancias del cigarro (Wu et al. 1997).

Considerando que el uso de diferentes materiales en la construcción del nido ha sido moldeado mediante selección natural, resulta interesante evaluar cómo los individuos responden a cambios en la disponibilidad de materiales como los filtros de cigarro, qué efecto tienen en la nidada, en los padres y, en consecuencia, en el éxito reproductivo. Es por esto que esta tesis está centrada en estudiar los efectos positivos y negativos, de la incorporación de filtros de cigarro en el nido.

I. CAPÍTULO I. “Becoming citizens: avian adaptations to urban life”

Resumen

Aunque las ciudades han existido desde hace algunos milenios, ha sido en los últimos siglos en los que se han expandido para convertirse en el rasgo dominante del paisaje. El crecimiento de las ciudades ha desplazado los hábitats originales y ha creado nuevos, obligando a las aves a ajustar su conducta, fisiología y sus historias de vida a las condiciones novedosas o a ser desplazadas a un paisaje rural, alterado y en constante disminución. Aquí identificamos las características más importantes –estructura del hábitat, estacionalidad, interacciones interespecíficas y la contaminación- que hacen que las ciudades difieran de los ambientes naturales y a las cuales las aves deben responder. Posteriormente, describimos algunas formas en las cuales las aves urbanas difieren de las que se encuentran en ambientes rurales. Finalmente, evaluamos si estas diferencias son adaptaciones a las condiciones urbanas o si son expresiones pre-existentes de adaptaciones a condiciones naturales, como plasticidad conductual, que también permitiría la colonización de ambientes urbanos.

Capítulo en prensa.

Chapter 6
Becoming Citizens: Avian Adaptations to Urban Life

Constantino Macías Garcia, Monserrat Suárez-Rodríguez, and Isabel López-Rull

Abstract Although cities have existed for some millennia, it has been only in the last few centuries that they have expanded to become a dominant feature of the landscape. Their growth displaces original habitats and creates new ones, facing birds with the challenge of adjusting their behaviour, physiology and life histories to the novel conditions or be displaced into a shrinking and also increasingly altered rural landscape. Here we identify the salient features—habitat structure, seasonality, interspecific interactions and pollution—in which cities differ from natural environments and to which birds must adjust. Then we describe the several ways in which urban birds have been found to differ from their rural counterparts. Finally, we evaluate whether these differences constitute adaptations to urban conditions or whether they are expressions of pre-existing adaptations to natural conditions, such as behavioural plasticity, which also permit the colonisation of urban habitats.

Keywords ■ AU1

6.1 There Was Once a Country Sparrow

Organisms constantly modify the environment, and it has been argued that virtually every trait they exhibit has consequences on other organisms (Bailey 2012). Indeed, our constant physical and chemical interactions with the surroundings, including the biota, influence in several ways the functioning of the ecosystems. While most such influences may be important, their impact on the environment is usually minor, yet some have major, even drastic environmental effects. The history of life on Earth provides several examples of massive effects of organisms on the

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6.2 How Are Cities Different from Natural Areas?

6.2.1 Habitat Structure and Seasonality

Cities represent a local change in habitat structure, climate and productivity, in which the adaptations of local organisms to the pre-existing natural environment may no longer work. Such disturbance alters ecological interactions thus leading to changes in the biological communities (Shochat et al. 2006). Both the identity and the distribution of plant species—sources of food, shelter, nesting places and materials for native birds—are different between the cities and their surroundings (e.g. McDonnell et al. 1997), and bird communities are responsive to those differences (Day 1995; Carbó-Ramírez and Zuria 2011; Becker 2013). Cities are not built to promote biodiversity; thus their plant communities include only a small number of species, often ornamental of exotic origin, which may not cover the needs of the local avifauna (but see Gleditsch 2016). Nevertheless, green islands of vegetation— parks and gardens— which receive regular irrigation, palliate some of the consequences of urbanisation. Urban gardens help to buffer the fluctuations of temperature and humidity due to the dryness and reflection coefficient (albedo) of concrete and asphalt surfaces (Gilbert 1989; Jauregui 1991). Gardens also provide birds with regular food supplies in the form of invertebrates that feed on the irrigated plants and thrive in the thermal conditions provided by the vegetation (Pickett et al. 2001). Finally, gardens offer shelter from the weather and predators to potential nesting places; it is in them that native and exotic avian species alike concentrate (Susca et al. 2011; Lugo et al. 2012). Urban gardens may also be frequented by predators, thus potentially acting as ecological traps (Sorace and Visentin 2007). Because of the constant irrigation, and also from design (as they include many perennial species), these urban green islands can also represent a buffer from seasonality.

AU3

Many organisms adaptively time several aspects of their life history to the predictable periodic changes that are caused by geophysical cycles (Lack 1968; Murton and Westwood 1977; Nicholls et al. 1988). In addition to internal rhythms, such tracking of the seasons occurs in response to changes in environmental variables (e.g. temperature, photoperiod, rainfall and food availability; Dawson 2008). In cities, however, seasonality is buffered, and the cues that birds use to track it may be blurred by gardening activities (Haggard 1990; Shochat et al. 2006). Also urbanisation could alter phenology through altered photic conditions due to artificial lights at night. Yet, reduced seasonality does not necessarily need to be bad for birds and may be the key to the success of some urban birds. For instance, bird assemblage composition in parks of Valencia is maintained through the constant arrival of migrant species through the year, arguably favoured by the stability of the conditions, including food availability (Murgui 2007). These circumstances favour resident species that can become dominant by excluding others from their urban park territories and also contribute to the lack of functional response, by which population size responds to fluctuations in food production.

For other species, however, becoming urban residents is not an option, either because they are migrants who need to reach their breeding or wintering grounds on time or because the seasonality of key elements of their ecology (e.g. food or predators) is not influenced by the conditions in the cities. Therefore, it is crucial for them to correctly read the changes in the season in order to not risk local extinction through the timing of food, shelter and other resources (Lugo et al. 2012).

6.2.2 *Interspecific Interactions*

Urbanisation modifies the structure of animal communities and the way in which organisms interact. Cities do harbour a much reduced diversity of vertebrate predators than natural environments (McKinney 2002). This is somewhat counterbalanced by the very high numbers of those predators that are found in cities, particularly domestic cats (*Felis catus*), whose reported effect on urban birds can range from minor (e.g. Gering and Blair 1999; Gillies and Clout 2003) to severe (e.g. Woods et al. 2003; Loss et al. 2013), often compromising the persistence of urban bird populations (e.g. Baker et al. 2005; Van Heezik et al. 2010). The latter may be particularly true for native bird species in areas where cats are not originally native (Sorace 2002). Predation by cats may influence the composition of the urban bird assemblage, with the least susceptible species becoming dominant (e.g. Noske 1998); it is intense, affecting millions of birds every year, although perhaps not more so than in nonurban environments (Baker et al. 2008), and it appears to afflict birds in low condition (old, diseased, injured), as would be expected elsewhere (Baker et al. 2008).

The responses of avian predators to urbanisation vary between species and as a function of the urbanisation process. Specialist predators, being more sensitive to changes in the prey community, are typically much less frequent in cities than in the surrounding habitats (Blair 2001; Mckinney 2006), which is not the case of more generalist predators (Sorace and Gustin 2009). It has been argued that since predator size is positively correlated with flight distance (from humans), large predators are less likely to colonise urban habitats, thus allowing some relative large prey species to settle there (Møller et al. 2012). This process would not apply to nocturnal predators, as human activity is much reduced at night (Chace and Walsh 2006; Sorace and Gustin 2009).

An impoverished community of large predatory birds (see Møller et al. 2012) appears to contribute to the settlement of large corvids in some cities (e.g. Vuorisalo et al. 2003). Corvids, in turn, are often responsible for high rates of nest predation in cities (Major et al. 1966). Other known nest predators such as snakes and small carnivores (i.e. viverrids or procyonids) are also scarce in most cities, which instead harbour unnaturally high concentrations of cats.

Th evidence of how urbanisation affects the interaction between birds and predators is not uniform, probably due to the fact that the planning of urban landscapes varies across cities, thus affecting the assemblage and functioning of

urban biological communities. Some authors report that predation decreases with urbanisation (e.g. Møller 2010), which is consistent with the fact that the density of breeding birds is higher in urban areas than in their surroundings (although the diversity of breeding species follows the opposite trend; Lancaster and Rees 1979; Beissinger and Osborne 1982; Shochat 2004). However, other works report that in the cities, there is a higher risk of predation, particularly on nests (Haskell et al. 2001; Sorace 2002; Jokimäki et al. 2005; Chace and Walsh 2006), which constitutes a crucial limiting factor for avian populations (Martin 1993; Conway and Martin 2000). Again, cats frequently prey on fledglings, and it has been argued that they have a major negative impact on breeding success of urban birds (Sorace 2002; Woods et al. 2003; Baker et al. 2008).

Because urbanisation brings about major changes in the composition of biological communities, it is reasonable to expect that it has an effect on parasite-/pathogen-host interactions. In the case of birds, this possible link has not been very extensively investigated (c.f. Delgado and French 2012), but available information suggests that the effect of urbanisation on the prevalence of avian parasitic infections is a function of the parasites' life cycle. On the one hand, both helminth parasite richness and prevalence (Aponte et al. 2014; Calegaro-Marques and Amato 2014) and diversity of blood parasites have been found to decrease with urbanisation, arguably due to loss of intermediate hosts (Fokidis et al. 2008; Geue and Partecke 2008). Conversely, viral infection (*Avipoxvirus*) and the severity of coccidial (*Eimeria* spp.) infection are positively associated with the degree of urbanisation in the house finch (*Haemorhous* [= *Carpodacus*] *mexicanus*; Giraudeau et al. 2014). These two pathogens are directly transmitted, and their prevalence may be linked to the high density of finches in the cities and possibly also to the abundance of bird feeders that promote contagion. This latter effect is worrying, since high densities of parasitised urban birds may spill infection to the wild bird living in the neighbourhood of cities (see Bradley and Altizer 2007).

It is unclear why intermediate hosts (mainly ectoparasites) should be less abundant in the cities than in rural areas, given that a large proportion of avian ectoparasites complete their life cycles in the bird nests (López-Rull and Macías Garcia 2015) or on their plumage, while small water deposits that act as breeding sites for *Diptera* (mainly mosquitoes) are normally abundant in urban areas. As indicated above, most studies suggest that the effect of urbanisation on avian parasite biology depends on the life cycle of the parasites, yet other factors such as pollution may mediate this link. For instance, Bichet et al. (2013) found that lead pollution, which is high in cities, is linked with prevalence of *P. relictum*. This protozoan is responsible for one type of avian malaria, and since it requires an intermediate (arthropod) host, it would not have been expected to be too prevalent in cities.

will review some examples of phenotype changes following urbanisation and the nature of them.

6.5 Adapting to Life in the Cities

6.5.1 Habitat Structure and Seasonality

Responsiveness to attributes that indicate seasonality varies amongst and between species and depends on phenotypic plasticity, which allows colonisation of different environments (Lambrechts et al. 1996). As we saw above, cities buffer the seasonal changes in the weather and in food availability. Cities also blur some of the cues that can be used by birds to adjust their physiology/behaviour to changes of the season. These changes should have led urban birds to become less seasonal. One demonstration that this is the case comes from studies showing increased sedentariness in urban blackbirds (*Turdus merula*; Partecke and Gwinner 2007). The authors looked at the pattern of nocturnal activity and fat deposition, two variables tightly linked to predisposition to migrate, of hand-reared birds from urban to nonurban localities and found that the former had a significantly reduced tendency to migrate. An earlier common-garden experiment by the same group (Partecke et al. 2004) demonstrated that, although the timing of reproduction was not different between birds from urban to nonurban areas, the former initiated earlier (both sexes) and finished also earlier (females) their reproductive hormonal activity, implicating a genetic difference in their physiological response to environmental cues (see also Partecke et al. 2005). As the authors recognise (Partecke and Gwinner 2007), these experiments do not quite preclude the possible influence of early developmental (e.g. maternal) effects, yet taken together they constitute one of the most convincing cases so far of adaptation by birds to urban life. As indicated above, light pollution is associated with the adaptive shift in the timing of breeding and sedentariness of urban blackbirds (Dominoni and Partecke 2015), yet other ecological variables are associated with life-history differences between city and field blackbirds in Spanish populations (Ibáñez-Álamo and Soler 2010). Since membership to different lineages suggests that blackbirds have invaded European cities several times independently (Evans et al. 2009), it is conceivable that different processes have led to subtly different adaptations to urban life in different cities, although to date only the case of the Munich population (Partecke et al. 2004; Partecke and Gwinner 2006) constitutes a demonstration of life-history adaptation [AU5] of blackbirds to life in the cities.

Other environmental variables may also drive changes in seasonality or migratory patterns of urban birds. For instance, regular food availability promotes earlier breeding in urban than rural populations of Florida scrub jays (*Aphelocoma coerulescens*; Schoech and Bowman 2001), possibly because it reduces the production of corticosterone, a hormone with negative effects on reproduction. The

toxicants and has generally a low ratio of nutritional to energetic content. This might have promoted adaptations in generalist urban birds to avoid less nutritious/more toxic food as well as physiological adaptations in food-supplied urban birds (such as hummingbirds which are commonly attracted to feeders) to cope with a diet rich in refined sugars.

Reports abound of birds starting to exploit anthropogenic food sources, from the opening of milk bottles by tits in Britain to the cracking of nuts using cars by Japanese crows (*Corvus macrorhynchos*; Nihei and Higuchi 2001). Blue (*Cyanistes caeruleus*) and great tits (*Parus major*) were originally found in 1921 to open milk bottles to consume the cream accumulating under the lid. At the time fresh milk was customarily delivered at the doorstep in glass bottles fitted with a flexible metal lid (tin and more recently aluminium). As bottles remained some time before being taken indoors, this provided opportunity for birds to approach, inspect and exploit this source of fat. The spread of this behaviour was mapped and quantified by Fisher and Hinde (1949; Britain) and Hinde and Fisher (1951; Europe). This enabled Lefebvre (1995) to evaluate the spread rate with models used in the study of human cultural transmission (Cavalli-Sforza and Feldman 1981). That bottle opening by Paridae was culturally transmitted which was not certain, particularly since Sherry and Galef (1984, 1990) demonstrated that American parids (*Parus* [= *Poecile*] *atricapillus*) are very likely to spontaneously open a bottle and that the probability that this happened was not influenced by the presence of an experienced tutor. Lefebvre (1995) found that accelerating (e.g. exponential) functions best described the spread of bottle opening by tits in Britain and in Belfast. This is consistent with the cultural transmission assumption of an autocatalytic increase in the rate of spread over time. The idea that cultural transmission is involved was further supported by the fact that data only poorly fitted the linear wave-of-advance model which would describe the spread of the behaviour as a function of a constant rate of untutored learning.

We devoted some time to this phenomenon as it is a textbook example of how birds adjust their behaviour to exploit anthropogenic food sources. The controversy regarding the mechanism of spread of milk bottle opening could be framed in the wider discussion of whether such examples are genuinely novel traits that reflect adaptation to cities or whether they represent exaptations (the expression in a novel context of a pre-existing adaptation to deal with similar conditions; in this case the adaptive behaviour of tits to uncover food items under lichens and bark). As seems to be the case, this exemplifies two different exaptations: a tendency to seek food under lichens and bark and a predisposition for cultural transmission. Also it illustrates the transient nature of cities and the challenges and opportunities they pose to colonising birds (and other organisms). The capability of learning—whether socially or otherwise—and thus adjusting the behaviour to novel conditions may be a key factor enabling some species to colonise urban environments (although we note that cognitive abilities such as problem-solving need not necessarily be better in urban than in rural populations; Papp et al. 2015). Maklakov et al. (2011) demonstrated that brain size was positively associated with the probability that members of passerine bird families, as well as individual species, bred in European

city centres. These comparative analyses were prompted by the idea that cities present birds with new challenges, and the previous demonstrations that big brains facilitate the colonisation of novel environments, and that this effect is due to the cognitive consequences of having a big brain (Sol et al. 2005). In their work, Sol et al. (2005) used, as proxy of cognitive ability, the number of foraging novelties reported for the species included in their study. This is a widely used index, yet it is not exempt of problems, for it relies on perfect knowledge of foraging strategies. For instance, the finding that *Parus* (= *Poecile*) *atricapillus* spontaneously opens bottles suggests that this is likely to be an exaptation rather than a genuine urban novelty. Still, employing behaviours evolved in one environment to exploit resources encountered in a novel, one may require a degree of behavioural plasticity. Since both technological and cultural changes ensure that the conditions confronted by urban birds change constantly, it is likely that some degree of behavioural plasticity is required to remain a successful urban coloniser. However, even if behavioural plasticity requires large brains, the reported link between brain size and urbanism (Maklakov et al. 2011) is as likely to be a consequence of differential colonisation of cities by large-brained species as it is of being the result of adaptation to urban life.

6.5.3 Interspecific Interactions

Nest predation influences both nesting strategy (cavity, open, etc.) and patterns of nest attendance (e.g. Conway and Martin 2000) and determines the nature of urban bird assemblages (Jokimati and Huhta 2000). But is there evidence that birds adapt to the particular predation regimes found in cities?

AU8

A first line of defence against predation is avoiding encounters with predators. Birds may achieve this by selecting safe/secluded perching, roosting and nesting places (see Marzluff 2001). We are not aware of adaptive differences in those behaviours between wild and urban bird populations; birds in forests and cities alike take readily to breeding in nest boxes, and birds nesting in building crevices are probably even safer than their rural, cliff-nesting counterparts.

Encounters with predators can also be avoided by preventing detection, for instance, through cryptic colouration. There is no suggestion, however, that the reported cases of difference in colour between urban and rural birds (e.g. Haag-Wackernagel et al. 2006; see above) are the consequence of difference in predation regimes. Also, although adaptation is suspected in the case of rapid evolution of geographic colour races of *P. domesticus* in North America, as it parallels the pattern of geographic variation of other species it is now sympatric with (Johnston and Selander 1964), there is no evidence that this is a response to predation, nor that it constitutes an adaptation to urban life.

Early detection of predators in the neighbourhood may allow the birds to either avoid the place altogether (e.g. Amo et al. 2015) or to monitor its behaviour and take evasive action as necessary. In birds, evasive action means to fly, and the distance at which a bird decides to fly is a function of both its fleeing tendency and of the cost of leaving the place (Blumstein 2006). When low-risk encounters are too frequent, tolerance would be advantageous, as it may allow birds to exploit feeding resources efficiently. Accordingly, Møller (2008) found that urban birds have a reduced flight distance than their rural conspecifics. Further, Møller (2009) found that rural populations of bird species that have successfully become city dwellers have shorter flight distances than sympatric populations of nonurban species. This suggests that short flight distance may preadapt species to life in the city, although the possibility that flight distance in rural populations has been influenced by gene flow from urban populations cannot be ruled out. Mutually supporting evidence strongly suggests that this is useful for city birds to have a short flight distances and that a short flight distances enable colonisation of urban habitats. Yet there is no evidence so far demonstrating that flight distance of a particular species has been reduced following colonisation of cities and that such modification is genetically based, i.e. short flight distance appears to be and exaptation, not an adaptation to life in the cities.

Urbanisation also affects the interaction between parasites and their hosts. One intriguing possibility is that cities provide some effective means for birds to deal with ectoparasites. Indeed, urban birds have been reported to collect discarded cigarette butts, rip them open and use the tar-loaded filter fibres as lining material for their nest, thus bringing about a reduction in the number of ectoparasites recovered from the nests (Suárez-Rodríguez et al. 2013). Since a similar effect is achieved by wild birds using green material from plants with known repellent activity (Dubiec et al. 2013), such behaviour may not be an adaptation to life in cities but a modification of the materials used for antiparasitic defence. This assumes that similar cues at the nest trigger the use of green aromatic plants and discarded cigarette butts and that similar cues are also used to find and gather both materials. On the other hand, the effectiveness of both aromatic plants and discarded cigarette butts as ectoparasites repellent has not been compared. It may be that the high concentration of substances in the smoked-through filters makes them more effective at repelling ectoparasites (and also toxic; Suárez-Rodríguez and Macías Garcia 2014), thus further promoting their use. The impact of urban nest materials on ectoparasites abundance and diversity, and hence on the prevalence of the pathogens they transmit, remains hypothetical.

6.5.4 Pollution

Pollution may, in principle, select for advantageous physiological traits to detoxify (chemical), adjust response thresholds (light) or modify the emission of signals to avoid masking (noise). Although chemical pollution has diverse impacts on bird populations, both within and outside the cities, we found no reports of novel adaptations to contend with it, but there is one possible case of an exaptation (Chatelain et al. 2015). The capture of zinc (and other heavy metals) by feather

melanin makes advantageous for birds to be dark in areas polluted with heavy metals. Urban pigeons have been found to be darker than their suburban counterparts (Chatelain et al. 2015), and this seems to represent an example of directional selection in cities of an attribute evolved earlier in the lineage of vertebrates. Work in this area is likely to become more common as avian genomic tools become more abundant (see Zhang et al. 2014), which will allow the characterisation of urban-rural differences, if any, on the expression of genes involved in detoxification.

Birds undoubtedly adapt to prolonged exposure to light in the cities, as they sleep through the night (albeit less than in wild conditions; see above). Yet this is likely sensory adaptation and is unlikely to be different from that undergone through the year by birds living at high latitudes. But this sensory adaptation is not complete, and as we saw above, hormonal activity, circadian rhythms, length of breeding season and onset of maturity are all affected by urban light patterns in a way that suggest that no evolutionary adaptation to light pollution is taking place.

Sound pollution in cities evokes a variety of responses from birds. When exposed to noise that may mask their vocalisations, birds can modify the song output (Díaz et al. 2011), increase the duration of their songs (Ríos-Chelén et al. 2013) or otherwise modify their structure (Francis et al. 2011), and they may be uttered at different times (Fuller et al. 2007) and/or have some frequency components altered (see reviews in Slabbekoorn 2013; Gil and Brumm 2013). A small but growing number of papers also report that some bird species can adjust in real time their songs in response to sudden bursts of noise (Bermúdez-Cuamatzin et al. 2009, 2010; Gross et al. 2010; Verzijden et al. 2010), whereas only a handful involve species which lack (or appear to lack) the capability to vary their songs (Francis et al. 2011; Ríos-Chelén et al. 2013). These later cases may be examples of adaptation to urban conditions by non-learning songbirds, although some degree of vocal plasticity in suboscine birds cannot be ruled out (e.g. Ríos-Chelén et al. 2005, 2012), and other mechanisms such as differential habitat use (discussed in both Francis et al. 2011 and Ríos-Chelén et al. 2013) could explain the difference in song attributes between urban and rural populations of non-learning bird species.

AU9

Halfwerk et al. (2011) provide perhaps the best case for the adaptive value of song adjustment to overcome masking of vocalisations by urban noise. They showed experimentally that although low-frequency songs are preferred by females and linked to female fecundity, exposure to urban noise impairs their effectiveness and favours higher-pitched songs. Yet, this convincing evidence of the fitness value of adjusting the frequency of song to prevent masking does not demonstrate adaptation to cities, because male great tits can adjust the frequency of their songs in real time (i.e. this is an adaptive plastic response, presumably evolved elsewhere, that is also useful in cities).

6.6 Conclusion

We have seen that most differences between rural and urban birds can be explained as being consequence of either phenotypic plasticity or of differential colonisation by individuals or species with sufficient behavioural plasticity to move into novel environments. These two patterns raise the question of whether cities in fact represent novel, more complex environments than birds would have experienced in their evolutionary past. It is possible, instead, that cities are as challenging as any novel environment that may confront birds, and the relative paucity of globally successful urban species is the result of the rapid overtaking of other environments by the cities. Still, it may be that the very speed at which urban landscapes spread at the expense of natural ones poses a particular novel challenge to would-be urban birds. We also saw that biodiversity within the cities, although lower than in their surroundings, is dominated by local species.

We argue that, in terms of complexity, cities cannot compare with forest ecosystems, particularly with those in the tropics. Cities do not have the vast numbers of species (and hence of potential interactions) of tropical forests, and their physical complexity is also probably lower. Seasonality is buffered in the cities, and many links to parasite transmission are therein disrupted. It is also unlikely that cities expose birds to physical or biological conditions that are genuinely novel, in the sense of not having being experienced by birds over their evolutionary past. Yet in one attribute, these environments pose a fundamentally different challenge, cities keep changing. The urban conditions that house sparrows confronted in the cities of the Fertile Crescent some 10,000 years ago are much more similar to current rural conditions than to anything the same species confronts in the midst of contemporary cities. Yet this urban species persists, suggesting that it has kept pace with urban change (but see below). Whether being possessed of a large brain has been the key to such behavioural plasticity is an open question. Another open question is whether the current urban species will persist, and the answer to this question seems to be negative in the case of one of the earliest city dwelling birds, the house sparrow currently declining in Europe (Summers-Smith 2003). Even as birds adapt—or adjust—to living in cities, the cities continue changing; waste management modifies every few human generations the distribution and availability of food, environmental awareness means that measures are taken to promote both the number and the diversity of species in our cities (e.g. green roofs and walls, Baumann 2006; Chiquet et al. 2013). As successful city dwellers become enormously numerous compared with their rural conspecifics, the rural environment shrinks. This may lead to a constant flow of inadequate phenotypes or maladaptive genes from the city to wild, which arguably may drive to extinction the rural populations and seal the destiny of such species that have colonised them, to that of the cities.

Finally, in spite of an already large and rapidly increasing number of works centred on potential bird adaptations to urban life, there is a dire need of studies that use in the cities the same tools traditionally used for detecting adaptation in nature,

the direct measure of fitness in relation to the putative urban-adapted traits, together with measures of its genetic underpinning.

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AU11

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II. OBJETIVOS

Objetivo General

Explorar la posible causa de la elección, así como las consecuencias, de utilizar filtros de cigarro para construir el nido.

Objetivos e hipótesis por capítulos

Capítulo II. "There is no such a thing as a free cigarette; lining nests with discarded butts brings short-term benefits, but causes toxic damage"

Cuantificar los costos y beneficios que experimentan los pollos al crecer en nidos forrados con filtros usados de cigarro.

Hipótesis y predicciones

La presencia de filtros usados de cigarro en el nido tiene consecuencias benéficas para las aves.

a) La tasa de crecimiento (peso y talla) de los pollos incrementará en función de la cantidad de filtros de cigarro incorporados en el forro del nido ya que éstos reducen el número de ectoparásitos presentes.

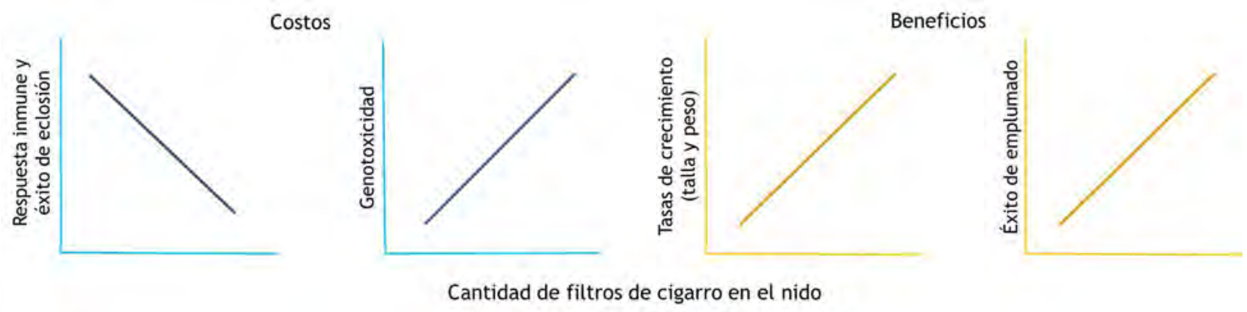

Figura 1. Representación gráfica de las predicciones sobre las consecuencias del uso de filtros de cigarro en los pollos que crecen en contacto con ellos.

El uso de filtros de cigarro en el nido tiene consecuencias negativas para las aves.

a) El éxito de eclosión y la respuesta inmune estarán negativamente relacionados con la cantidad de filtros de cigarro.

b) La cantidad de daño genotóxico sufrido por los pollos -medido en sus eritrocitos- se asociará positivamente con la cantidad de filtros de cigarro en los nidos (Figura 1).

Capítulo III. “Anthropogenic nest materials may increase breeding costs for urban birds”

Evaluar el daño genotóxico causado por el uso de filtros de cigarro, en adultos reproductores de gorrión inglés y pinzón mexicano. Determinar si el nivel de exposición a los filtros de cigarro durante la reproducción determina la cantidad de daño genotóxico.

Hipótesis y predicciones

En *Carpodacus mexicanus* la construcción del nido la llevan a cabo las hembras, el macho solo vigila y tiene poco contacto con el nido. Las hembras tienen contacto con el

nido durante casi toda la temporada reproductiva, desde el tejido del nido (que efectúan con el pico), la incubación (durante la cual pasan día y noche en el nido), y hasta la crianza de los pollos (cuando solo están en el nido para alimentar a las crías). En *Passer domesticus*, ambos padres construyen, incuban y crían a los pollos. Por lo tanto:

Los padres que construyen nidos con filtros de cigarro sufren consecuencias negativas debido a la manipulación de los filtros de cigarro, y éstas pueden variar con el grado de contacto que tengan con los filtros.

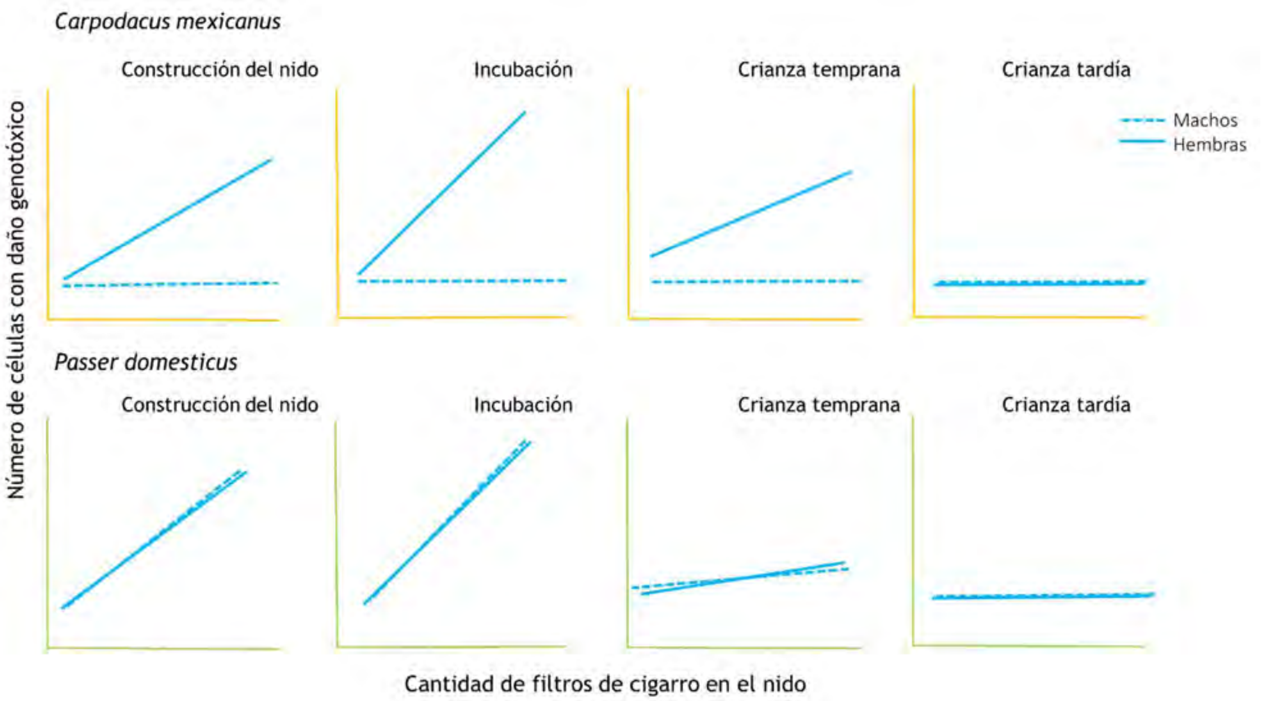

Figura 2. Predicciones sobre la genotoxicidad de aves adultas que incorporan filtros de cigarro a sus nidos. Esperamos que el efecto sea dinámico debido a la magnitud del contacto con el material de filtros.

a) El daño genotóxico será mayor cuando haya un contacto prolongado con el material del nido.

b) Las hembras experimentarán mayor daño genotóxico que los machos debido al contacto constante que tienen con los filtros de cigarro.

c) Al no tener mucho contacto con el nido, los machos de *C. mexicanus* tendrán un menor daño genotóxico que las hembras. En *P. domesticus*, machos y hembras tendrán el mismo grado de daño genotóxico, ya que ambos participan de manera similar en las actividades reproductivas (Figura 2).

Capítulo IV. "An experimental demonstration that house finches add cigarette butts in response to ectoparasites"

Determinar si las aves agregan filtros de cigarro a sus nidos en respuesta a incrementos en la cantidad de ectoparásitos.

Hipótesis y predicciones

Las aves ponen en sus nidos fibras de celulosa de filtros usados de cigarros en respuesta a la presencia de ectoparásitos en sus nidos.

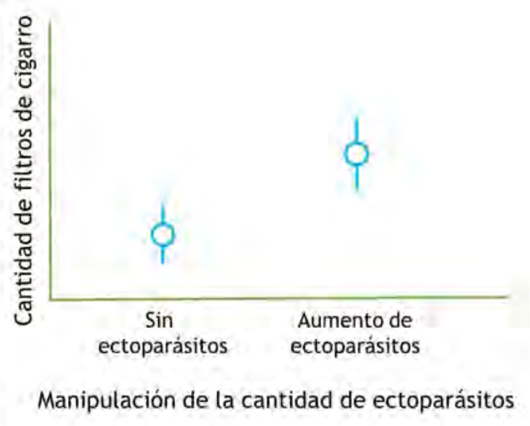

Figura 3. Esperamos que las aves agreguen filtros de cigarro a sus nidos cuando aumentemos la carga de ectoparásitos.

a) Al incrementar la carga de ectoparásitos, las aves agregarán filtros de cigarro a sus nidos (Figura 3).

III. CAPÍTULO II. “There is no such a thing as a free cigarette; lining nests with discarded butts brings short-term benefits, but causes toxic damage”

Resumen

La adaptación a los ambientes modificados por los humanos, como las ciudades, es y será un componente muy importante de la historia natural de muchas especies. Las aves han mostrado adaptaciones en sus vocalizaciones, el uso de sitios de anidación y en sus ritmos de actividad en los ambientes urbanos. Anteriormente reportamos que algunas especies como el pinzón mexicano (*Carpodacus mexicanus*), utilizan filtros de cigarro como material de nido, y esto se relaciona con la disminución de ectoparásitos en el nido, probablemente porque la nicotina funciona como repelente de artrópodos. La nicotina es solamente una de las sustancias peligrosas que contiene el cigarro. En este estudio, investigamos si la presencia de estos químicos afecta al pinzón mexicano. Encontramos que el éxito de eclosión y de emplumado, así como la respuesta inmune de los pollos se relacionan positivamente con la proporción de filtros de cigarro en el nido. Sin embargo, encontramos signos de genotoxicidad en las células de la sangre asociados con la cantidad de material de filtros de cigarro en el nido. Aunque desconocemos los efectos de la genotoxicidad en la supervivencia de post-volantón y en el éxito reproductivo, parece que los filtros de cigarro en los nidos tienen consecuencias negativas que pueden balancear los beneficios de su uso como repelente de ectoparásitos.

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There is no such a thing as a free cigarette; lining nests with discarded butts brings short-term benefits, but causes toxic damage

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fledging;
genotoxicity;
nests materials;
urban birds.

Abstract

Adaptation to human-modified environments such as cities is poised to be a major component of natural history in the foreseeable future. Birds have been shown to adapt their vocalizations, use of nesting places and activity rhythms to the urban environments, and we have previously reported that some species, including the house finch (*Carpodacus mexicanus*), use cellulose from smoked cigarette butts as lining material and thus reduce the number of ectoparasites in their nests, probably because the nicotine repels arthropods. Nicotine is only one of hundreds of potentially harmful substances found in cigarette butts. Here, we investigated whether the presence of such chemicals is harmful for house finches adding cigarette butts to their nests. We found that hatching and fledging success and chick immune response were all positively correlated to the proportion of the nest that was made up of butts. However, the signs of genotoxicity in the blood cells also increased with the proportion of butt cellulose in the nests. Although we have not measured the effect of genotoxicity on post-fledging survival and breeding success, it seems that bringing cigarette butts to the nest has negative consequences that may counterbalance the benefits of using them as ectoparasites repellents.

Introduction

A rapidly expanding field in Evolutionary Ecology is the study of how – and which – organisms adapt to urban environments (Alberti *et al.*, 2003). Besides being the newest significant environments on earth, cities differ in many key attributes from natural environments (Bowman & Marzluff, 2001; Marzluff, 2001), particularly in the absence/looseness of feedback loops between the demography of interacting species (notably between the demography of consumers and food production). One of the questions for which there is not, as yet, a unified answer, is which attributes determine that some (most?) species are excluded from cities, whereas others thrive not only in the original cities they colonized, but seemingly in any city where they are subsequently introduced (Gering & Blair, 1999; Luniak, 2004; Ditchkoff *et al.*, 2006; Møller, 2009). Conditions in the cities may match the natural history of some species, or, more likely, species may colonize cities by adapting their behaviour/ecology to the new conditions (Jokimäki & Huhta, 2000; Ditchkoff *et al.*, 2006). Work on birds has uncovered vocal adaptations to urban noise (Slabbekoorn & Peet, 2003; Bermúdez-Cuamatzin *et al.*, 2009; Ríos-Chelén *et al.*, 2012), use of human-made structures as nesting places (Blair, 2004; Møller, 2009), and adjustments of the activity budgets and reproductive rhythms (Fernández-Juricic & Tellería, 2000; Fleischer *et al.*, 2003).

Reproductive adaptations, such as building nests in the right places and using the correct nest materials, are a prerequisite for the successful occupation of habitats by birds. In addition to buffering changes in temperature and humidity (Collias & Collias, 1984; Hansell, 2000), bird nests provide shelter against predators and may also include some protection against

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ectoparasites, such as green leaves of aromatic plants that act as arthropod repellents (Clark & Mason, 1988; Ontiveros *et al.*, 2007; Mennerat *et al.*, 2009). Appropriate plants may not be available in cities, where at least the house finch (*Carpodacus mexicanus*), and the common house sparrow (*Passer domesticus*) line their nests with cellulose from smoked cigarette butts and as a consequence reduce the number of nest ectoparasites (lice and ticks; Suárez-Rodríguez *et al.*, 2013). This may be because nicotine, found in the smoked butts, is a secondary metabolic compound of tobacco plants, which probably evolved it as an adaptation to combat herbivory (Baldwin & Preston, 1999; Strauss & Agrawal, 1999; Adler *et al.*, 2006). However, unlike plants with secondary metabolites, filters from commercial cigarettes contain in excess of 400 substances, many of which are known to be toxic (Wu *et al.*, 1997; Pascual-Villalobos, 1998). Adult birds would be exposed to such toxicants when manipulating the butts to extract the smoked-through cellulose fibres, and both adults and chicks would also be exposed when sitting on top of these fibres (hens would be additionally exposed when preening during incubation and brooding). Given that nestlings' exposure to stressors has long-term consequences on bird lifespan (e.g. Monaghan *et al.*, 2011), it is likely that such exposure to butts toxicants is harmful to the birds. Here, we evaluated whether the reduction in nest parasites that results from the use of cigarette butts promotes fitness, and whether being exposed to toxic substances in the filters results in toxic damage that could counter such hypothesized benefits (Suárez-Rodríguez *et al.*, 2013).

To evaluate the fitness consequences of lining nests with discarded cigarette butts, we recorded clutch size, hatching success, fledgling success and growth rate of first clutches of house finch (*C. mexicanus*) nesting in Mexico City and assessed their possible associations with amount of cellulose from cigarette butts and of ectoparasites in the nests, that we quantified using the methods developed by Suárez-Rodríguez *et al.* (2013). As an additional measure of condition, we assessed the immune response (reaction to phytohaemagglutinin; Gwinner *et al.*, 2000) of 13-day-old chicks. Finally, we quantified the number of nuclear abnormalities in red blood cells (micronuclei, nucleoplasmic bridges, nuclear buds and bi-nucleated cells), which are the result of genotoxicity leading to chromosomes breaking either in anaphase or during cytokinesis (Fenech, 1993, 1997, 2007; Phillips & Arlt, 2009).

Materials and methods

We included nests from three areas (S1 S3) with varying degree of urbanization, located around facilities within the main campus of UNAM (Ciudad Universitaria; México City). Starting in mid January 2013 (one month before the expected onset of nest building), each site was visited every five days. During the visits, we identified the potential nesting locations and followed the progress of the breeding activities until laying began ($n = 89$ nests built and 87 clutches). Then, we quantified the number of eggs laid and their hatching success (No. of eggs hatched/clutch size). At the first visit after hatching, the chicks' right tarsi were individually marked with nontoxic acrylic paint (colour blue, yellow, green was randomly assigned to each chick). During the subsequent weeks, we recorded the weight (g) and tarsus length (mm) of as many chicks as possible, ensuring that at least one chick was measured always (measuring all chicks was not always compatible with minimizing the stress for chicks and parents). Only data from this regularly measured individuals were entered in the analyses ($n = 74$).

When chicks were ca. 8 days old, we punctured the right brachial vein of the regularly measured chicks and collected 2 μL of blood in heparinized capillary tubes. Samples were kept and transported to the laboratory within 5 h. Then two smears were prepared from each sample, each using 1 μL of blood. These were fixed with methanol for 10 min, allowed to dry overnight and exposed to Schiff staining, which involves an oxidation of nucleotides and thus colours the nuclei and any segment of DNA present. Smears were then counter-stained with haematoxylin and observed under the phase-contrast microscope (Axiostar; Carl Zeiss Vision Inc., Toronto, ON, Canada) @ 1200X. One thousand red cells of each smear were characterized as either normal, containing micronuclei, nucleoplasmic bridges, nuclear buds or as being bi-nucleated; the last four conditions being taken as evidence of genotoxicity. Only 66 regularly measured chicks were included as some chicks could not be reached at the specified age.

When chicks were between 13 and 15 days old, we measured the width of both left and right patagia (wing webs) of regularly measured chicks, then inoculated phytohaemagglutinin (PHA 0.4 mg 0.04 mL of PBS buffer) to one randomly-selected patagium and 0.04 mL of PBS (PBS Buffer) to the other wing as a control. We measured both patagia again 24 h later and used the increase in thickness as a measure of the magnitude of the immune response to PHA (only $n = 62$ chicks could be inoculated and recaptured within 24 h). All measures were taken three times, and the average value entered in the analyses. The first 38 chicks were measured with a Truper™ (Jilotepec, México) dial calliper (± 0.02 mm) and the remaining 24 with a Mitutoyo™ (Aurora, IL, USA) micrometre (± 0.001 mm). Nineteen of those measured with a micrometre were measured also with the calliper, and we found that the two methods gave the same measure of the magnitude of the immune response ($r = 0.96$, $P < 0.0001$; see Fig. S1).

At the end of the season, we collected all the nests in individual plastic bags. In the laboratory, they were

processed following the same protocol used in 2011 by Suárez-Rodríguez *et al.* (2013). Briefly, each nest was placed in a Berlese-Tullgren funnel to collect and quantify all the ectoparasites that it contained (Newell, 1955; Sandler *et al.*, 2010). These were kept in containers (one per nest) with 70% ether until counted under the stereoscopic microscope (Zeiss™ Stemi, Carl Zeiss Vision Inc., Jena, Germany). We then un-weaved the nests, separated and weighed the different materials that made it.

Statistical analyses

We evaluated whether, as in 2011, the number of ectoparasites in the 2013 nests was a function of the proportion of the nest weight that was made of cellulose from smoked cigarettes (hereafter amount of cellulose), using a generalized linear model with a declared Poisson distribution. We included site and cellulose as possible explanatory variables and selected the model according to the Akaike information criterion (AIC). Weekly changes in chick weight were used to calculate growth rates using linear models, with proportion of cellulose in the nest as potential explanatory variable. As our estimate of age was imprecise, we included tarsus length as a co-variable to control for differences in growth rate due to differences in size (age) at which chicks were measured. Again, we selected the best model using the AIC.

Then, we used general linear models (GLMs) to determine whether exposure to toxic substances embedded in the cellulose from smoked cigarettes affects hatching (as egg shells are permeable: Marco *et al.*, 2004) and fledgling success. Explanatory variables were the amount of cellulose from butts, site, laying date and either clutch size (for hatching success) or brood size (for fledging success). For both response variables, we declared a binomial distribution with a logit link and selected the best model according to the AIC.

We used the total number of cells with genotoxic signals (cells with micronuclei, nucleoplasmic bridges, nuclear buds or bi-nucleated) out of 2000 erythrocytes as the dependent variable in generalized linear Poisson models to evaluate whether this was a function of the amount of cellulose from cigarette butts. We included site and size measures (chick weight and tarsus length at the time when the sample was taken) as potential explanatory variables and selected the best model following the AIC. Immune response was assessed by first comparing (with a *t*-test) the increase in thickness in both patagia to ensure that the swelling in the wing inoculated with PHA was a measure of the intensity of the immune response, then applying linear models to determine whether there was an association between the intensity of the immune response and the amount of cellulose from cigarette filters in the nest. We used site and size (chick weight and tarsus length at the time of the immune challenge) as potential explanatory variables. As before, we selected the best model according to the AIC.

Finally, a structural equation modelling (SEM) analysis was used to explore the (potentially causal) direct and indirect links between the several variables measured. This type of analysis is useful to explore relationships between several variables that explain a dependent variable of interest (Klem, 2000). We entered as an independent variable the amount of cellulose from cigarette butts in the nest and as a dependent variable the weight of the regularly measured chick at fledging. We retained the models that maximized the comparative fit index (CFI) and minimized the root mean square error of approximation (RMSEA), also ensuring that the observed data did not differ significantly from the model predictions (X^2).

Results

One model best explains the variance in number of ectoparasites; it indicates that the number of ectoparasites recovered from the nests, is a locally varying negative function of the amount of cellulose in the nest (Fig. 1), thus mirroring the results of our previous study. Tarsus growth rate was explained only by the size at the beginning of measures and by site, whereas weight gain, was a positive function of amount of cellulose from butts ($\beta = 0.851$) and a negative function ($\beta = -0.015$) of initial weight (Fig. 2; the best ten models are shown in Table S2). The amount of cellulose from butts was positively linked to hatching success (Table S3), and it was explained by site according to the model with the lowest ΔAIC (Fig. 3; Table 1).

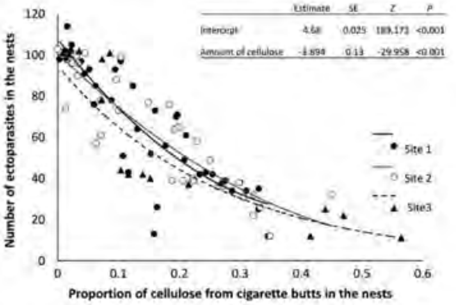

Fig. 1 The number of parasites recovered from the nests was a negative function of the proportion of the nest materials that was made up by cellulose from discarded smoked cigarette butts. Although there were significant differences between sites, the model was additive (i.e. not interactive), meaning that the direction of the effect was the same in all sites.

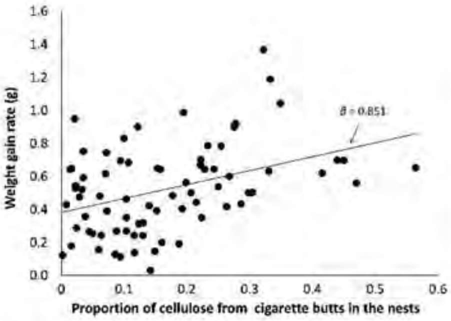

Fig. 2 Chick growth rate, expressed as the weekly increase in weight, was as positive function of the proportion of the nest that was made up of cellulose from cigarette butts. The regression line corresponds to that from the linear model (see text).

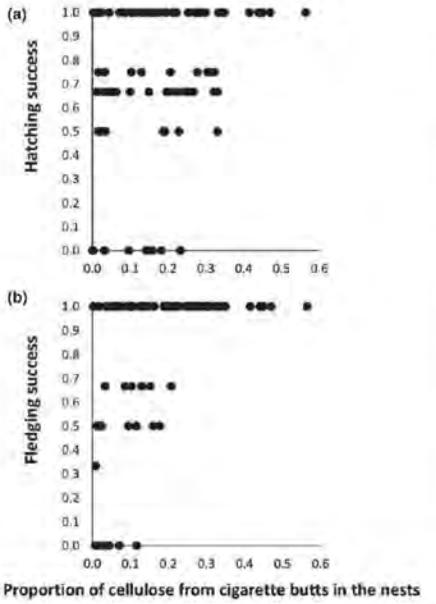

Fig. 3 Nests with greater egg (a) and chick survival (b) were built with larger proportions of cellulose from discarded cigarette butts. The significant models are described in Table 1.

Amount of cellulose was also positively associated with fledging success, which was negatively associated with brood size (Fig. 3; Table 1: note that this function varied with site).

The number of nuclear abnormalities in the red blood cells, a measure of genotoxic damage, was a positive function of the amount of cellulose from smoked cigarettes found in the nests (β = 2.459). Genotoxicity was a negative function of size (β = −0.044) and was associated with site (Fig. 4; the best ten models are shown in Table S4). The magnitude of the immune response was a positive function of amount of cellulose from butts (β = 1.042) and a negative function of weight (β = −0.007; Table 2) according to the model with the lowest AIC (see the ten best models in Table S5).

The structural equation modelling produced one best model (*AIC* = 380.087: Fig. 5). This includes only significant regressions and its predictions fitted the data ($X^2_3 = 5.114$, $P = 0.244$; $CFI = 0.99$; $RMSEA = 0.019$). According to the resulting path, the greater the amount of cellulose from cigarette butts, the fewer ectoparasites that are retrieved from the nest and the greatest the evidence of genotoxic damage; ectoparasites load in turn negatively affects the weight of fledglings, whereas genotoxic damage is positively related to the immune response, which positively affects the final weight of fledglings (see Fig. 5).

Discussion

Our data confirm with a new, larger sample, the negative association found earlier (Suárez-Rodríguez *et al.*, 2013) between the amount of cellulose fibres from cigarette butts used to line the nests of *C. mexicanus* and the number of ectoparasites retrieved from the nests at the end of the season. Ectoparasites are known to influence bird fitness (Lehmann, 1993; Brown *et al.*, 1995; Proctor & Owens, 2000), and in particular, mites and ticks are known to decrease bird survival (Proctor & Owens, 2000). With high metabolic demands and often competition by nest mates, chicks are likely to be very vulnerable to haematophagous parasites (Brown *et al.*, 1995; Merino & Potti, 1995; Fitze *et al.*, 2004) and a reduction of parasite infestation in the nest should promote survival. Whereas we found no effect of the amount of butt cellulose on the rate at which the tarsi grew, we found that it had a substantial effect on the mass gain. The models explaining both growth rates included the original measure, as larger (older) chicks should have reduced growth rates. As weight at fledging is a mayor predictor of post-fledging survival (e.g. Naef-Daenzer *et al.*, 2001), there is an obvious advantage in gaining weight rapidly, and growing in a nest with few (particularly haematophagous) parasites – because of the amount of butt cellulose used to line it – is likely to promote weight gain. Given the positive effect on survival, this suggests that there is a short-term benefit of using cigarette butts to line the nests, but this may involve a costs later in life. We found that the amount of cellulose from cigarette butts in the nest

Table 1 Estimates from the best models that explain hatching (amount of cellulose – site) and fledging success (brood size – amount of cellulose).

	Hatching success					Fledging success			
	Estimate	SE	Z	P		Estimate	SE	Z	P
Intercept	0.465	0.329	1.457	0.145	Intercept	0.341	0.868	0.393	0.694
Amount of cellulose	3.571	1.371	2.604	0.01	Brood size	−0.204	0.361	−0.566	0.571
Site 1	0.274	0.346	0.792	0.428	Amount of cellulose	18.164	3.328	4.431	< 0.001
Site 3	0.555	0.489	1.135	0.256					

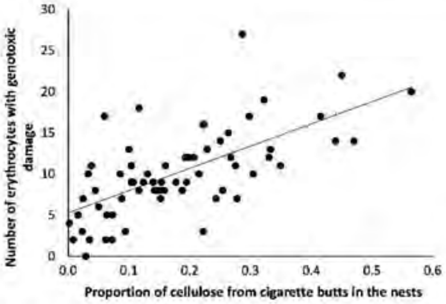

Fig. 4 The proportion of cellulose from cigarette butts that made up the nest was positively correlated with the total number of erythrocytes with evidence of genotoxic damage (see text). The regression line is shown to aid interpretation, but the significant model (Table S4) was built declaring a Poisson distribution.

Table 2 Estimates from the best model to explain immune response (amount of cellulose – weight).

	Estimate	SE	t	P
Intercept	0.467	0.1	4.673	< 0.001
Weight	0.007	0.006	1.139	0.259
Amount of cellulose	1.042	0.182	5.731	< 0.001

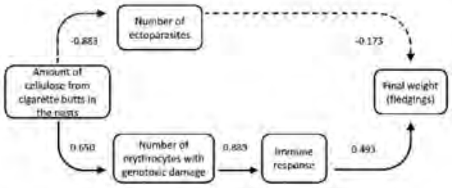

Fig. 5 Path analysis diagram showing the indirect and direct effects between amount of cellulose from butts in the nests (independent variable), the number of nest ectoparasites, the measure of genotoxicity (see text), the magnitude of immune response (to phytohaemagglutinin) and the weight of the chicks at fledging (response variable). Standardized regression coefficients are indicated next to each path.

is positively associated with the probability of hatching and of fledging. It is unclear why the smoked-through cellulose fibres promote hatching success, but one possibility is that they reduce the negative effects of nest ectoparasites on sitting hens (which may even abandon infested nests; Oppliger *et al.*, 1994).

One reason to measure the immune response of chicks in this study was that nicotine has been found to be an immunosuppressant in humans (Sopori, 2002). Yet we found that the amount of cellulose from cigarette filters was positively associated with a robust immune response induced by PHA, of a magnitude comparable to that reported using a similar protocol on chicks of the same age and species (Navara *et al.*, 2006). If a strong immune response is an indication of health, this result may be consequence of a reduced exposure to ectoparasites (which would deteriorate the general condition of chicks). Alternatively, the immune system of the chicks may have been sensitized by the exposure to cigarette toxic substances, leading to a strong reaction to PHA. Indeed, Gwinner *et al.* (2000) found that starlings (*Sturnus vulgaris*) have an increased response to PHA when using green plants in their nests, and the authors attributed this to the presence of some substance(s) in plants that stimulate the immune system. If – as it is likely – the arthropod-repellent effects of cigarette butts and of green plants added to nests are homologous, it would not be surprising that their effects on the bird's immune system is also homologous.

Genotoxic damage induces chromosomal breaks – for instance through impairment of cytokinesis (Fenech, 1993) – and is a frequent consequence of oxidative stress (Risom *et al.*, 2005). The greater the amount of cellulose from cigarette butts in the nest, the more erythrocytes showed signs of genotoxicity. The level of erythrocyte damage that we found in butt-exposed chicks (mean ± SD: 1.3 ± 0.96 ‰ micronuclei, 1.91 ± 1.33 ‰ binucleated cells, 1.09 ± 0.79 buds and 0.74 ± 0.87 nuclear bridges) is larger than the reported frequency of micronuclei in bird erythrocytes from 30 species (0.12 ± 0.21, range 0–1.06 ‰; Zúñiga-González *et al.*, 2000), but this report is based mostly on captive (zoo) and domestic birds, each species represented by only 4 ± 3.1 (range 1–13) individuals of unknown (or unreported) history/condition and includes only two Passeri-

formes. Our data also indicate greater damage than that found by Skarphedinsdottir *et al.*, (2010) in much larger (and longevous) free-living herring gulls (*Larus argentatus*) exposed to urban pollution (range 0.18 0.28 ‰), thus we are confident that our data indicate that during the stay at the nest, the substances that are embedded in the smoked-through cellulose are interfering with cell division in growing Mexican finch chicks. We have no information on the likelihood that this damage may have long-term negative effects such as reduced survival in short-lived passerine birds, yet nestling exposure to stress hormones which promote oxidative stress and thus genotoxic damage (Risom *et al.*, 2005) does reduce lifespan in captive zebra finches (*Taeniopygia guttata*; Monaghan *et al.*, 2011). Additionally, there are anecdotal reports of cage birds being intoxicated and subsequently dying following ingestion of cigarette butts (Novotny *et al.*, 2011), as well as experimental evidence that exposure to cigarette butts causes mortality in other short-lived vertebrates (e.g. fish such as the fathead minnow *Pimephales promelas*; Slaughter *et al.*, 2011). Also, inasmuch as damage has to be repaired, birds may have to pay later in life the use of valuable resources (e.g. antioxidants; Blount *et al.*, 2001; Barja, 2002; Cohen *et al.*, 2007) to contain genotoxic damage. In male *C. mexicanus*, this may impair carotenoid-based ornament expression and hence mating success (Hill & Montgomerie, 1994; McGraw *et al.*, 2001).

Our study is correlational, and thus our inferences about causality should be regarded as provisional. However, the application of structural equation modelling (SEM) allows the comparison of several models that is path diagrams to generate robust causal explanations (Mitchell, 1992) which may later be tested experimentally. Following such SEM approach, we were able to trace the consequences for *C. mexicanus* of using cellulose from cigarette butts to line their nests. Butts deter (possibly because the nicotine they contain) and may also harm (due to the other chemicals in them) ectoparasites. Butts also generate genotoxic damage. We counted damage in erythrocytes, yet there is no reason to suppose that this is not a reflection of toxic damage in other tissues (MacGregor *et al.*, 1997). The increased toxicity was associated to an increase in the immune response, and although the causal link is unknown it is possible that substances in the butts are both genotoxic and stimulants (sensitizers) of the immune system (see above). As this increase in immune response was equivalent to that reported in a study that applied a comparable protocol to chicks of the same species and age (Navara *et al.*, 2006), we suspect that the correlated increase in genotoxicity was not due to a heightened immune sensitivity producing autoimmune reactions. Finally, both the reduced number of parasites and the enhanced immune response are linked to fledging weight a correlate of survival (Magrath, 1991; Naef-Daenzer *et al.*, 2001; Suedkamp Wells *et al.*, 2007). Although important links are missing, we propose that this pathway is an accurate representation of the consequences of incorporating cigarette butts in nests by urban birds. Still, the full fitness consequences of this behaviour can only be known once we have evaluated the negative long-term consequences, such as a reduced expression of male ornaments or an accumulated toxic damage in females following repeated breeding attempts.

Use of cigarette butts in nests seems to be an adaptation to urban life that reflects, as most adaptations, a trade-off between short-term benefits and long-term costs. Life-history theory predicts that attributes of this type should be promoted because of the greater demographic impact of early vs. late reproduction (Stearns, 1989). However, urban ecology is far from invariant, and widespread adaptations to cities which may entail a loss of genetic variance may rapidly become obsolete or even turn maladaptive, a problem that may increasingly face urban biota.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Correlation between measures of patagia made with a dial calliper and with a micrometer for the immune response test.

Table S1 Results from the *t*-test to evaluate the difference (increase) in thickness between the wing inoculated with PHA and the wing inoculated only with PBS buffer.

Table S2 Best ten models to Tarsus growth rate (left) and weight gain (right) as functions of amount of cellulose, site and initial size.

Table S3 Best ten models to explain hatching (left) and fledging success (right) as functions of amount of cellulose, laying date, clutch size and site.

Table S4 Best ten models to explain genotoxic damage as a function of amount of cellulose in the nest, site, chick size (tarsus length) and chick weight.

Table S5 Best ten models to evaluate the effect of amount of cellulose, chick size and weight, and site, on the magnitude of the immune response (inflammatory reaction to PHA).

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IV. CAPÍTULO III. “Anthropogenic nest materials may increase breeding costs for urban birds”

Resumen

Aún en especies socialmente monógamas, el conflicto sexual es una de las razones que promueve diferencias en los roles sexuales durante la reproducción. Esto puede provocar que uno de los sexos realice una contribución desproporcionadamente grande, y por lo tanto experimente costos muy altos en momentos particulares de la época reproductiva. En la Ciudad de México, algunas especies de aves agregan fibras de filtros de cigarro a sus nidos, lo que reduce la cantidad de ectoparásitos, pero también aumenta el daño genotóxico. Los machos de *Passer domesticus* realizan una contribución sustanciosa en la construcción del nido mientras que los machos de *Carpodacus mexicanus* no contribuyen a esta actividad, por lo que pensamos que los efectos tóxicos de la exposición a los filtros de cigarro debe de ser mayor para las hembras de *C. mexicanus* que para los machos conespecíficos, pero no debe haber diferencias entre sexos en *P. domesticus*. Como esperábamos, encontramos más daño genotóxico en los glóbulos rojos de las hembras que incuban de *C. mexicanus* relacionado con la cantidad de filtros que había en el nido y además era mayor que el de sus machos conespecíficos. El daño en los macho no estaba asociado con los filtros de cigarro; inicialmente fue menor que el daño de las hembras, pero incrementó cerca del momento de volantones, momento en el que los machos alimentan a las crías. En ambos padres de *P. domesticus*, sin embargo, el daño genotóxico

se presentó de manera similar y se relacionó con la cantidad de filtros de cigarro en el nido. El uso novedoso de un material tóxico, que funciona como repelente de ectoparásito, provoca costos en la época reproductiva para el miembro que invierte más en la construcción del nido y la incubación.

Capítulo en prensa.

Anthropogenic Nest Materials may Increase Breeding Costs for Urban Birds

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Author contribution statement

CMG and MSR designed the study. MSR collected the data, performed the analyses, and generated an initial draft of the report. CMG provided logistical support and edited the manuscript. RDMM advised on the measure of toxicological damage, which was conducted in her facilities. All authors reviewed and approved the final version.

Keywords

Cigarette butts, Genotoxicity, Carpodacus mexicanus, passer domesticus, parental care, sex roles

Abstract

Word count: 238

Even in socially monogamous species, sexual conflict is one reason that often promotes differences in the roles of sexes during reproduction, which may lead to one sex making a disproportionate contribution, and thus incurring disproportionate costs, at particular moments of the breeding process. In Mexico City, a number of songbird species line their nests with fibers from discarded cigarette butts, which reduce ectoparasite load but are genotoxic. As male Passer domesticus make substantial contributions to nest building whereas male Carpodacus mexicanus do not contribute to nest building, we hypothesized that the toxic effects of exposure to cigarette butts should be greater for females C. mexicanus than for conspecific males, but that there should be little or no difference in P. domesticus. As expected there was more exogenous genotoxic damage in the red-blood cells of incubating female C. mexicanus the more cigarette butts were found in their nest, and much more than in their conspecific males. Damage in males was not associated to cigarette butts; it was initially lower than in females, but it increased near fledging, together with their breeding effort. In both male and female P. domesticus, however, genotoxic damage was equally apparent and greater the more cigarette butts were in the nest. The novel use of a toxic, anthropogenic parasite repellent by urban birds may be thus asymmetrically increasing the breeding costs paid by the member of the pair most involved in nest building and incubation.

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Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: Yes

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The study was designed, and birds were handled, in accordance to the Guidelines for the treatment of animals in behavioural research and teaching published by Animal Behaviour (DOI: DOI:10.1016/j.anbehav.2011.10.031). This report has been approved by an ad-hoc ethical committee set up by the Programa Universitario de Bioética (UNAM).

Anthropogenic Nest Materials may Increase Breeding Costs for Urban Birds

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Abstract

Even in socially monogamous species, sexual conflict is one reason that often promotes differences in the roles of sexes during reproduction, which may lead to one sex making a disproportionate contribution, and thus incurring disproportionate costs, at particular moments of the breeding process. In Mexico City, a number of songbird species line their nests with fibres from discarded cigarette butts, which reduce ectoparasite load but are genotoxic. As male *Passer domesticus* make substantial contributions to nest building whereas male *Carpodacus mexicanus* do not contribute to nest building, we hypothesized that the toxic effects of exposure to cigarette butts should be greater for females *C. mexicanus* than for conspecific males, but that there should be little or no difference in *P. domesticus*. As expected there was more exogenous genotoxic damage in the red-blood cells of incubating female *C. mexicanus* the more cigarette butts were found in their nest, and much more than in their conspecific males. Damage in males was not associated to cigarette butts; it was initially lower than in females, but it increased near fledging, together with their breeding effort. In both male and female *P. domesticus*, however, genotoxic damage was equally apparent and greater the more cigarette butts were in the nest. The novel use of a toxic, anthropogenic parasite repellent by urban birds may be thus asymmetrically increasing the breeding costs paid by the member of the pair most involved in nest building and incubation.

Key-words: cigarette butts, genotoxicity, *Carpodacus mexicanus*, *Passer domesticus*, parental care, sex roles

Introduction

Human activities impact the environment in a variety of ways, often with negative consequences for the local biota (Miller and Hobbs, 2002). This is clearly the case with urbanization, where organisms are exposed to a new array of stressors that impose substantial constraints on their biology (Ditchkoff et al., 2006). Behavioral modifications are amongst the first responses of animals to life in the cities (Baldwin, 1896; Price et al., 2003). This is because behaviour can be speedily adjusted through learning, thus providing a rapid means to adapt to new conditions (Ditchkoff et al., 2006; Sih, 2013; Sih et al., 2011). For instance, the plasticity of Passeriformes' song production (Slabbekoorn, 2013) allows them to make instantaneous adjustments of frequency in response to sudden increases of anthropogenic noise, as has been experimentally shown by Bermúdez-Cuamatzin et al., (2010) and Gross et al., (2010). Other examples include short-term increases in competitive behaviour of invasive crabs (Tanner et al., 2011) and changes of time budgets in birds near airports (Gil et al., 2014).

Animals may modify behaviourally to an array of human disturbances. Thus the negative effects of habitat fragmentation may be contended with by adjusting dispersal and foraging patterns (Hovland et al., 1999; Rathcke and Jules, 1993; Schtickzelle et al., 2006; Tscharntke and Brandl, 2004). For instance, the bog fritillary butterfly (*Proclossiana eunomia*) show a dispersal depression in fragmented landscapes, as dispersal between landscape fragments is linked to high mortality (Schtickzelle et al., 2006), whereas root voles (*Microtus oeconomus*) forage preferentially on the edges of fragmented landscape patches (Hovland et al., 1999). Human alteration of the environment often involves the translocation of organisms. For instance, urban biota is increasingly comprised of non-native species, which may negatively interact with native organisms. Consequently, prey species often have to adapt their behavior to the presence of invading predators, whereas competing local species may need to escalate competition to reduce the negative effects of such introductions (Sih et al., 2010). Equally insidious is the effect of invasive species on the local species mating systems (e.g. Valero et al. 2008).

Humans also modify the environment by polluting it. In particular, anthropogenic chemicals may interfere with the expression of sexual traits such as coloration, courtship or singing performance (Gorissen et al., 2005; Markman et al., 2008) partly because they often act as hormonal disruptors, which may provoke changes in the operational sex-ratio, thus interfering with mating behavior (Fry, 1995). A complex form of pollution is that caused by solid waste. This is composed of discarded traces of products used by humans. A sometimes large fraction of the consumables that we use is discarded; it is mostly unfriendly with the environment and may take a long time to degrade. Several types of solid waste are harmful to humans, animals and the ecosystems (Hamer, 2003). Human-generated waste is ubiquitous in cities and elsewhere, and, as it often resembles natural materials, it is frequently used by mammals (Cavia et al., 2009) and birds (Townsend and Barker, 2014; Votier et al., 2011; Wang et al., 2009) in the construction of their nests. Since the distribution and physical properties of waste products is not the same as those of natural materials, the use of waste products requires behavioural adjustments, such as the ripping-of and unweaving of cigarette butts to line nests (Suárez-Rodríguez et al 2013). Given the paramount influence of the nest materials on the microclimate and the biota to which developing offspring are exposed (Collias, 1964; Hilton et al., 2004), incorporating anthropogenic materials on the nests is likely to have a major influence on the natural history of those using them (Antczak et al., 2010; Hartwig et al., 2007; Suárez-Rodríguez et al., 2013; Wang et al., 2009).

Eggs and chicks are directly in contact with the nest lining; a thin soft structure of feathers, fur and the like. Often, it also includes green plant materials that serve to repel ectoparasites (e. g. in the common starling *Sturnus vulgaris*; Clark and Mason 1987; Clark and Mason 1988) as they contain secondary metabolites evolved to prevent herbivory (Wink, 1988; Wittstock and Gershenzon, 2002). In other species such as blue tits (*Cyanistes caerules*), green materials seem to act against bacteria in the skin of chicks (Mennerat et al., 2009a) which is linked with faster mass gain (Mennerat et al., 2009b). Note, however, that the same materials may be added for reasons unrelated to egg/chick wellbeing. For instance, in spotless starlings (*S. unicolor*) the addition of green plant material to the nest serves mainly as a sexually selected signal that stimulates the production of testosterone in females, and may also signal male status (Polo et al., 2010; Veiga et al., 2006). Some bird species use this strategy to repel arthropods from their nests (Gwinner and Berger, 2006; Ontiveros et al., 2007; Wimberger, 1984) thus it has been considered a way of actively protecting the nest from ectoparasites (Clark and Mason, 1988). Bird ectoparasites are a well-known to be harmful, especially those occupying their nests (Clayton et al., 2010; Hund et al., 2015; also see López-Rull and Macías Garcia, 2015).

In addition to natural materials, two common city birds, *P. domesticus* and *C. mexicanus* weave anthropogenic materials (Van Riper III, 1976), including cotton fibres from discarded cigarette filters into the nest lining (Suárez-Rodríguez et al., 2013). Cigarette butts have a repellent effect similar to that of green materials (Suárez-Rodríguez et al., 2013), yet they also contain many toxic substances in addition to Nicotine, such as ethylphenol, heavy metals (e.g. titanium dioxide), propylene glycol, diverse insecticides, and even cyanide (Jensenl et al., 2004; Moriwaki et al., 2009; Register, 2013; Slaughter et a., 2011; Witschi et al., 2002). Thus chick erythrocytes show signs of genotoxic damage proportional to the amount of cigarette butts used to line their nests (Suárez-Rodriguez and Macias Garcia, 2014), probably as the toxic substances enter their bodies through their skins, which are in contact with the nest materials. Although we cannot exclude the possibility that at least those that are volatile are also inhaled. Genotoxic damage occurs when an exogenous or endogenous agent (including reactive oxygen species) breaks DNA chains during mitosis, a process that can lead to mutagenicity and eventually to carcinogenic processes (DeMarini, 2004; Fenech, 2007; Valko et al., 2004). DNA damage results in nuclear abnormalities, with cells containing a nucleus plus one or more micronuclei, or bi-nucleated cells with bridged nuclei. Damage to the mitotic apparatus (microtubules and microfilaments) results in true bi-nucleated cells (with a duplicated genome; Fenech 2007). While some damage may go undetected upon visual inspection, standardized counts of cells with nuclear abnormalities can be used as direct measures of genotoxic damage (Fenech, 1997).

Contact with discarded cigarette butts is toxic to chicks (Suárez-Rodríguez and Macías Garcia, 2014), and perhaps also to adults. Thus, by lining their nests with such materials, urban *P. domesticus* and *C. mexicanus* may be exposing themselves to a novel breeding cost, which we expect to differentially affect males and females given their unequal share of the breeding activities. Here we conducted standardized counts of damaged erythrocytes to quantify the cost incurred by male and female *P. domesticus* and *C. mexicanus* at different moments in their breeding season (stages). We hypothesized that the amount of direct contact with cigarette butts through manipulation and incubation should be positively correlated with genotoxic damage, and should thus differ between sexes and species in accordance to their breeding roles. We tested the above on the house finch and the house sparrow. These species coexist in North American cities, where they occupy similar ecological niches (Bent and Austin, 1968; Lowther and Cink, 2006; Woods, 1968),

to the point that competition often occurs between them (Bennett, 1990; Kalinoski, 1975; McClure et al., 2011).

Methods

Study species

Carpodacus mexicanus (Müller 1776), a native finch from North America (see Figure 1), is a socially monogamous bird with well-defined sexual roles and sexual dimorphism in color -adult males displaying bright carotenoid-dependent red crowns, bibs and rumps- as well as a large song repertory. Females are less conspicuous and choose males for their color and their songs (Nolan and Hill, 2004). Females are more actively involved in parental care than males, at least initially. They choose the nest site, build the nest, incubate the eggs and participate in the feeding of the chicks, whereas males feed the female during the incubation and do most of the feeding of fledglings, often also taking over the feeding of pre-fledging chicks (Hill, 2002).

Passer domesticus (Linnaeus 1758), the ubiquitous house finch (see Figure 1), is a socially monogamous European finch long naturalized in the Americas. It is also sexually dimorphic. Male ornaments (maroon and grey crown, black patches around beak and the eyes, a black bib, and dark back and wings that contrast with their pale underparts) are due to melanin, while females lack contrasting patches and seem drabber. Parental care is shared between sexes: both members of the pair choose the nest site, build the nest, incubate the eggs (female more actively) and feed the chicks (male more actively; Voltura et al. 2002; Hoi et al. 2003; Schwagmeyer et al. 2005; Liker et al. 2008).

Study site and blood samples

We conducted this study in a mixed colony of *C. mexicanus* and *P. domesticus* at the main campus of the Universidad Nacional Autónoma de México (UNAM) in the south of Mexico City. These species were breeding in natural conditions, constructing their nests on building structures or in trees. We worked with a mixed-species colony where nests are placed in close proximity (from 30 cm to 200 cm). Here, pairs of both species interact with each other throughout the breading season, feeding and collecting nest materials from the same places. Discarded cigarette butts are available on the ground, and are locally abundant at particular spots around the gates of the surrounding buildings. In the spring of 2014 we monitored the activity of the colony with binoculars (Minox™ BV 10 x 42) to assess the breeding stage and the number of eggs or chicks. We captured both members of each pair by setting mist nets for three days at the end of each of four 15-days intervals starting 15 days after the majority of the pairs had begun nest building. We could not recapture both members of the pair at all breeding stages, and this was declared in our statistical models. Thus we sampled breeding adults at end of a) nest building, b) incubation, c) early (chicks ≤ 15 days old) and d) late breeding (chicks ≥ 16 days old). Rodnan et al. 1956 reported that after 30-40 days radioactively marked erythrocytes were no longer present in pigeon (*Columba livia*) chick (*Gallus domesticus*) and duck (*Anas platyrhynchos*) blood samples, and from the reported curves it can be inferred that between 40-50% erythrocytes are removed from the bird's circulating blood every two weeks (see Reddy et al., 1975) for comparable results). Because of their higher metabolic rate (see Speakman, 2005) blood turnover is likely to be faster in smaller species, thus we decided that sampling every 15 days should provide a fair estimate of the condition of the birds (see below) in the preceding 2-3 weeks. We recorded the species, sex and size (tarsus length [mm] and weight [g])

and took a blood sample (~5µl) from each bird in a heparinized capillary tube after puncturing the brachial vein. Birds were marked with individual combinations of coloured plastic rings and one metal ring with a unique number. We identified the nest of each pair with the aid of binoculars and visited each nest to check attendance by the parents and to record the clutch and brood size. Only one of the 58 nests followed was deserted, and it happened before egg-laying (the nest is not included in the analyses). The remaining 57 nests were followed until their chicks fledged. Within 12 hours of collection we took the blood samples to the laboratory where we prepared two smears of each individual, to which we applied Schiff staining and counterstained with Hematoxylin. A coverslip was fitted with Entellan resin to each smear, which was inspected under a phase contrast optic microscope (Carl Zeiss™ Axiostar at a 1000X). We assessed 1000 erythrocytes from each smear (two smears per individual), counting every instance of a cell with micronuclei, nuclear buds, bridges in binucleated cells, and binucleated cells. At the end of the season we collected each focal nest, removed its parasites with a Berlese-Tullgen funnel, and separated and weighed all its component materials (Suárez-Rodríguez et al., 2013). We obtained the proportion of cigarette butts by dividing the butts weight between the total weight of the nest.

Statistical analyses

We captured and recaptured 41 parents from 26 nests of *Carpodacus mexicanus* and 55 parents from 31 nests of *Passer domesticus*. We analysed separately the two species, applying one generalized linear model to each, to determine which of our independent variables explained the number of blood cells with genotoxic damage (declaring a Poisson distribution for our dependent variable) using Rx64 s 3.1.2 software. Factors included were breeding stage (four levels), sex (two levels) and weight of cigarette butts in the nest (continuous variable).

After looking at the results we detected that there was an increment in the genotoxic damage experienced by males during the last two breeding stages. At this point, males make the bulk of the contribution to the feeding of the chicks. So, we explored whether the genotoxic damage was actually different from that experienced by females (see results and Figures 2 and 4) applying generalized linear models using only the data from the last two stages (early breeding and late breeding) and declaring a Poisson distribution for our dependent variable. We selected the best model using the Akaike Information Criterion (AIC) and ΔAIC, with ΔAIC <2 indicating that two models were equally supported by the data.

Results

Carpodacus mexicanus

We obtained two models that best explained the number of erythrocytes with evidence of genotoxicity (see supplementary Table S1). The best model was a three-way interactive model of the three potentially explanatory factors (proportion of cigarette butts, sex and breeding stage). The amount of cigarette butts in the nest was positively associated with the level of female, but not of male, genotoxic damage (Table 1). This effect was evident during nest building, and during incubation; it was much less pronounced during early- (Figure 2), but again very substantial in late breeding (Table 1; Figure 2). Thus the level of genotoxicity experienced by females during breeding is both large and linked to the amount of cigarette butts in the nest (Table 1; Figure 2).

Males had fewer red-blood cells with signs of genotoxic damage, and their number was consistently independent of the amount of cigarette butts incorporated into the nests. However, the

number of damaged cells increased through the breeding stages and appeared to be larger than the damage in females at the last breeding stage (Table 1; Figure 2). We evaluated whether breeding effort could explain the increase in male genotoxic damage (for instance through endogenous oxidative stress) by running a new model-selection procedure restricted to the early and late breeding stages, and incorporating brood size as a novel predictive factor. The model that best explains the amount of genotoxic damage includes an interaction between sex and brood size. It demonstrates that males attending larger broods had evidence of more genotoxic damage than males attending smaller broods (Figure 3). Female genotoxic damage was unrelated to brood size (Table 2; Figure 3).

Passer domesticus

The number of cells with evidence of genotoxic damage in *P. domesticus* was best explained by a three-way (sex, cigarette butts and stage of breeding) interactive model (Table S4). In this case, damage in both males and females during nest construction and incubation seemed positively influenced by the amount of cigarette butts in the nest (Table 3; Figure 4). However, during nest building, incubation and early breeding females had more cells with evidence of genotoxic damage than males. As we expected, the association between amount of cigarette butts in the nest and genotoxic damage was lowest in the last two breeding stages, when parents no longer occupy the nest. However, males had more genotoxic damage than females at the late breeding stage. (Table 3; Figure 4).

The number of erythrocytes with signs of genotoxic damage in males increased above the number of such cells in females during late breeding, and it was not related to the amount of nest cigarette butts. Consequently, we tested whether, as in *C. mexicanus,* this increase could be explained by endogenous stress related to the effort of feeding chicks. We thus ran the same model-selection procedure looking for a differential effect of brood size on genotoxic damage in male and female parents. Males showed more evidence of genotoxic damage than females during early and late chick breeding, but this damage was not associated with brood size in either sex (Table 4; Figure 3).

Discussion

We found fluctuating levels of genotoxic damage –measured as the number of red-blood cells with nuclear abnormalities- in breeding *P. domesticus* and *C. mexicanus* of both sexes. In females, at least during the early stages of the breeding attempt (nest building and incubation) such damage can be expressed as a function of the amount cigarette butts that they use to build their nest, and thus reveals a novel breeding cost for birds nesting in cities. This effect is evident in female *C. mexicanus*, which are in contact with the material during nest building and incubation, and in both members of *P. domesticus*, as they share the activities that lead to exposure with cigarette butts. Our results might be explained as a consequence of differences in health or condition between sexes or species. Yet this would not explain the significant link between the amount of butts in the nest and the numbers of erythrocytes with evidence of genotoxic damage; this was found in the sex most exposed to cigarette butts, and was found in the period when it is in a prolonged, direct contact with them. Since foraging is not restricted to areas where cigarette butts are abundant, we have no evidence of sex-specific diets in these species, and given that butts are never so copious that they

may substantially leak toxicants into the ecosystem, we think that our results cannot be explained as a result of species/sex differences in diet.

We argue that nuclear abnormalities reveal physiological costs because they are the result of genotoxic damage, and thus can be a proxy to estimate oxidative stress (Bartsch, 2002; Valko et al., 2004) such as that generated by exposure to toxic substances (DeMarini, 2004; Fenech, 1993; López Gordillo, 2012; Skarphedinsdottir et al., 2010; Valko et al., 2004). In addition to revealing damage, abnormalities in the nuclei of erythrocytes can inform approximately when it took place. This is because birds filter their blood through the spleen in cycles of approximately 15-30 days (Rodnan et al., 1956), thus between one half and the whole of the red-blood cells are replaced approximately every two weeks, which is similar to the duration of each of the breeding stages that we defined, and hence the span of time between our consecutive samples. Therefore, our measure of genotoxic damage and our sampling program allow us to explore the consequences of exposure to discarded cigarette butts during particular moments of the breeding cycle, when the relative commitment of males and females to the breeding effort may vary.

Our data not only reveal a novel breeding cost for urban birds, but show these costs to be unequally shared by both sexes. Male and female *C. mexicanus* play different roles in parental care (Badyaev and Hill, 2002; Duckworth et al., 2003b). Females build the nest, which means they, and not the males, manipulate all the materials that go into its construction. They also perform all the incubation (Badyaev and Hill, 2002; Duckworth et al., 2003a; Hill, 2002), thus being virtually continuously in contact with the nest materials. Accordingly, we found that not only females had more evidence of damage than males, but the number of nuclear abnormalities in their blood was tightly correlated with the amount of cellulose fibres from cigarette butts in their nests (= amount of cigarette butts). By contrast, during the same stages the males had very few damaged erythrocytes, and their numbers were independent of the amount of butts that went into the nest construction. This is evidence that a novel, anthropogenic nest material can generate breeding costs that are a function of the sexual differences in parental roles.

Males of *Carpodacus mexicanus* were apparently unaffected by the amount of cigarette butts in their nests, but they also showed evidence of genotoxic damage, particularly later in the breeding attempt. In *C. mexicanus*, males deliver food to the nest (Badyaev and Hill, 2002); this does not bring them into direct contact with nest-lining but there could be a certain amount of exposure to the toxic material, thus inducing variable amounts of genotoxicity. Furthermore, providing food is a demanding activity, particularly after hatching and increasingly so as fledging approaches. Exertions such as this can raise the level of oxidative stress, and we propose that this is the explanation of the increase in the number of nuclear abnormalities in male erythrocytes in the last two breeding stages (Figure 2), and of the apparent association of genotoxic damage with brood size (Figure 3).

Data from *P. domesticus* were also consistent with our prediction that exposure to cigarette butts promote genotoxic damage in breeding birds. In this case, however, the association between cigarette butts and nuclear abnormalities during nest building was indistinguishable between males and females (Figure 4). This may be due to the fact that male and female house sparrow participate in the construction of the nest and in the incubation off eggs (Bartlett et al., 2005). That the association was weaker in males during incubation is consistent with their reduced share in this activity (Figure 4). Thus, as in *C. mexicanus*, we uncover a breeding cost resulting from the use of anthropogenic nest materials by urban birds. Furthermore, we confirm that the extent of such cost is linked to the roles played by each pair member during the breeding effort.

In females of both species damage to the red-blood cells was reduced during early and late breeding, and in female house sparrows it was unrelated to the amount of cigarette butts that went into the making of the nest. Nor were nuclear abnormalities in females related to the number of chicks being raised, but we have little statistical power to detect such an effect.

Females of *C. mexicanus* show a late-breeding increase in genotoxic damage, which is linked, once more, to the amount of cigarette butts in the nest. We studied the genotoxic damage of birds raising their first brood, but since our population of *C. mexicanus* make several successive breeding attempts every year, and given their reported practice of clutch overlap (Evenden, 1957), we propose that the cigarette butt related increase in female nuclear abnormalities during late breeding is the consequence of renewed exposure to the nest lining due to incubation of a new clutch (as we systematically monitored the colony, we were able to detect clutch overlap). However, females do not build a new nest, but rather lay new eggs in the old one, thus exposure to butts, and hence genotoxic damage, are only moderately increased at this time. Indeed, we have witnessed clutch overlap within a nest, starting as early as when the chicks were approximately 5 days old.

The nests of *C. mexicanus* and *P. domesticus* contain the same proportion of cigarette butts (Suárez-Rodríguez et al., 2013), yet there appears to be more genotoxic damage in the former (see supplementary materials). If confirmed, this pattern could be the consequence of male and female sparrows sharing, and thus diluting, the effect of exposure to cigarette butts, yet in both species the extent of damage is similar during early breeding, but lower in house sparrows than in finches (compare Figure 2c and 4c). Another possibility is that this interspecific difference indicates that *P. domesticus*, with a long urban history that should have brought it into contact with anthropogenic pollutants, may have developed adaptations such as enhanced enzymatic activity (e.g. Rainio et al. 2012), that increase its ability to detoxify itself (Schwagmeyer and Mock, 2003). This tantalizing possibility would constitute one of very few cases of phenotypic adjustments to cities that may not be explained just a consequence of plasticity, but as genuine adaptation to urban life (see Suárez-Rodríguez et al. in press).

While we are confident that our measures of nuclear abnormalities are indicative of genotoxic damage, we have no inkling on what the long term consequences of this damage may be. Indeed, there is a paucity of works on the effects of toxicity on the longevity of short-lived Passerine birds. To be of consequence, genotoxicity –the damage of genetic material during cell division- should result in mutagenesis, which involves a modification of the genetic code that is transferred to the next generation of cells (Fenech, 2008). As we worked with cells that are constantly being produced and replaced (Rodnan et al., 1956), it may be that even if exposure to cigarette butts results in mutagenesis, this may not have time to express itself before the cell carrying it being discarded, unless the damage took place in erythropoietic stem cells, which would continue producing damaged erythrocytes. We counted nuclear abnormalities in erythrocytes because 1) their short life span allowed us to track the possible association between breeding activity and damage, and 2) extracting small amounts of blood to make two smears is both easy and relatively little intrusive. Still, damage may be occurring to other cell lineages -notably in the germ line- and in tissues or organs where it may have a more consequential effect on the condition, life expectancy and fertility of birds.

We have uncovered genotoxic consequences of breeding activities of birds using anthropogenic nest materials, and show that the amount of damage is linked to the roles played by both sexes during breeding. Cigarette butts repel nest parasites (Suárez-Rodríguez et al., 2013),

presumably because they contain nicotine (Harvey et al., 2007), and arsenic (Slaughter et al., 2011) which have been used as insecticides, as well as polycyclic aromatic hydrocarbons and other metals which could be responsible for the genotoxic damage experienced by both chicks (Suárez-Rodríguez and Macias Garcia, 2014) and adults (this work). Birds in nature also utilize some particular (plant) materials to deter parasites off the nest (Brouwer and Komdeur, 2004; Clark and Mason, 1988; Gwinner and Berger, 2008; Lafuma et al., 2001; Ontiveros et al., 2007; Polo et al., 2010), and it is conceivable that some of the substances involved (Gwinner and Berger, 2006) are also harmful to the birds. Still, both the diversity and the concentration of substances in discarded, smoked-through cigarette butts are likely to be much higher than those found in plants brought to the nests for prophylaxis, and we propose that the costs reported here apply mainly, if not only to birds using cigarette butts to line their nests. On the other hand, the ubiquitous application of insecticides and herbicides to crops, and their subsequent leaking into non-agricultural habitats, should make the exposure of birds to toxicants from nest materials a global reality, with consequences on the costs paid by the parents in accordance to their respective parental roles. This increasing risk of exposure to anthropogenic pollutants could elevate the cost of nest-building and incubation, with potentially negative demographic consequences.

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Ethical statement

In conducting this research, we adhered to the guidelines for the use of animals in research. We minimized handling stress, kept the sample to the minimum necessary to test the working hypotheses, and verified that no mortality happened as a result of our manipulations. This report has been approved by an ad-hoc ethical committee set up by the Programa Universitario de Bioética (UNAM).

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Figure legends

Figure 1. Female (far left) and male *Carpodacus mexicanus* (left), female (right) and male (far right) *Passer domesticus* (drawings by MSR).

Figure 2. During nest building (a) and incubation (b), genotoxic damage (expressed as the number of cells with nuclear abnormalities per 2000 erythrocytes) was associated in female *C. mexicanus* with the amount of cigarette butts incorporated into the nest. Variables are represented in the original scale to facilitate interpretation, but data were analysed using generalized linear models.

Figure 3. Genotoxic damage in erythrocytes of adult *C. mexicanus* (top) and *P. domesticus* (bottom) of both sexes during early and late breeding, as a function of the brood size (see text).

Figure 4. During nest building (a) and incubation (b), genotoxic damage (expressed as the number of cells with nuclear abnormalities per 2000 erythrocytes) was associated in female and in male *P. domesticus* with the amount of cigarette butts incorporated into the nest. Variables are represented in the original scale to facilitate biological interpretation, but data were analysed using generalized linear models.

Table 1. Comparison of effects of cigarette butts, sex and breeding stage on the amount of genotoxic damage (number of red-blood cells with nuclear abnormalities per 2000 cells) in *C. mexicanus*. Coefficients[1] come from the best supported model (see Table 1 of supplementary materials). Model = amount of cigarette butts × sex × breeding stage. Intercept = amount of damage at the onset of the breeding stage; Slope[1] = steepness of the association between amount of cigarette butts in nest and genotoxic damage; * = significant difference between sexes. (An analysis in which each nest is represented only by one if the parents, and only on one breeding stage, yielded essentially the same results; see Supplementary Materials Tables S8 and S9).

Breeding moment	Intercept		Slope	
	Females	Males	Females	Males
Nest building	1.656	1.652	5.241*	1.523
Incubation	1.983*	-0.501	4.657*	1.842
Early breeding	1.668	1.347	2.788	3.666*
Late breeding	-1.563	2.189*	12.335*	2.454

[1] Both intercepts and slopes in the table are composite (added) values of different coefficients (β) in the full model (see explanation in supplementary materials Table 3).

Table 2. Results of the best model that explains the amount of genotoxic damage in erythrocytes of male *Carpodacus mexicanus* at the last two stages of reproduction (early and late breeding, entered together in the analysis so as not to atomize the sample size). Model = sex × brood size; females and one-chick broods are coded as zero, hence their contribution is included in the intercept.

Variables	Coefficient	SE	Z	*P*
Intercept	1.569	0.204	7.685	< 0.001
Sex: males	0.118	0.281	0.420	0.675
Brood size: 2 chicks	-0.429	0.286	-1.502	0.133
Brood size: 3 chicks	-0.182	0.247	-0.739	0.460
Sex: males x brood size: 2 chicks	0.689	0.373	1.846	0.065
Sex: males x brood size: 3 chicks	0.710	0.326	2.178	0.029

Table 3. Comparison of effects of cigarette butts, sex and breeding stage on the amount of genotoxic damage (number of red-blood cells with nuclear abnormalities per 2000 cells) in *P. domesticus*. Coefficients[1] come from the best supported model (see Table 4 of supplementary materials). Model = amount of cigarette butts x sex x breeding stage. Intercept = amount of damage at the onset of the breeding stage; Slope = steepness of the association between amount of cigarette butts in nest and genotoxic damage; * = significant difference between sexes. (An analysis in which each nest is represented only by one if the parents, and only on one breeding stage, yielded essentially the same results; see Supplementary Materials Tables S10 and S11).

Breeding moment	Intercept		Slope	
	Females	Males	Females	Males
Nest building	1.997*	0.954	2.696	4.709
Incubation	2.001*	0.271	4.009	5.614
Early breeding	1.688*	0.918	-1.060	-0.261
Late breeding	0.844	2.148*	-0.811	-0.294

[1] Both intercepts and slopes in the table are composite (added) values of different coefficients in the full model (see explanation in supplementary materials Table 6).

Table 4. Results of the best model that explains the amount of genotoxic damage in erythrocytes of male *Passer domesticus* at the last two stages of reproduction (early and late breeding entered together in the analysis so as not to atomize the sample size). Model = sex + brood size; females and one-chick broods are coded as zero, hence their contribution is included in the intercept.

Variables	Coefficient	SE	Z	*P*
Intercept	1.276	0.120	10.668	<0.001
Sex: males	0.279	0.119	2.335	0.020
Brood size: 2 chicks	0.173	0.123	1.403	0.161
Brood size: 3 chicks	0.204	0.267	0.762	0.446

Figure 1.TIF

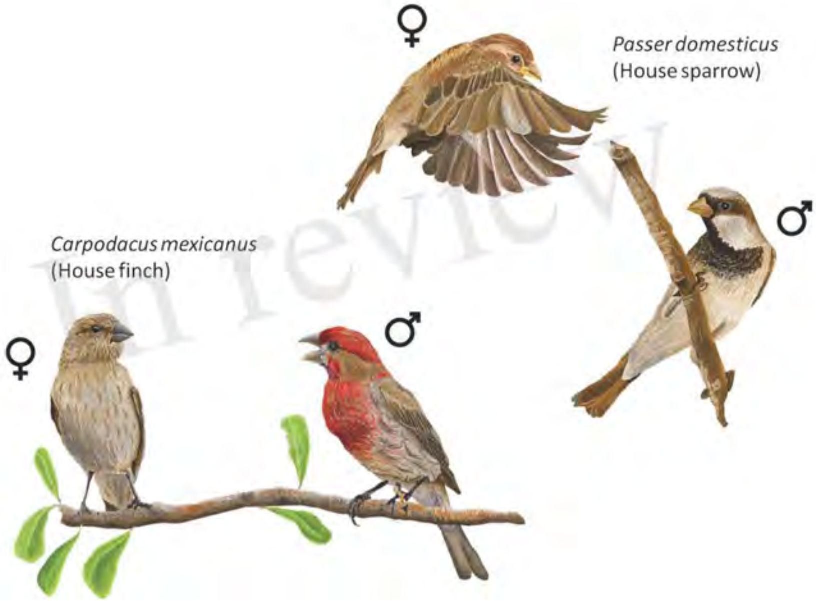

♀
Passer domesticus
(House sparrow)
♂
Carpodacus mexicanus
(House finch)
♀
♂

Figure 2.TII

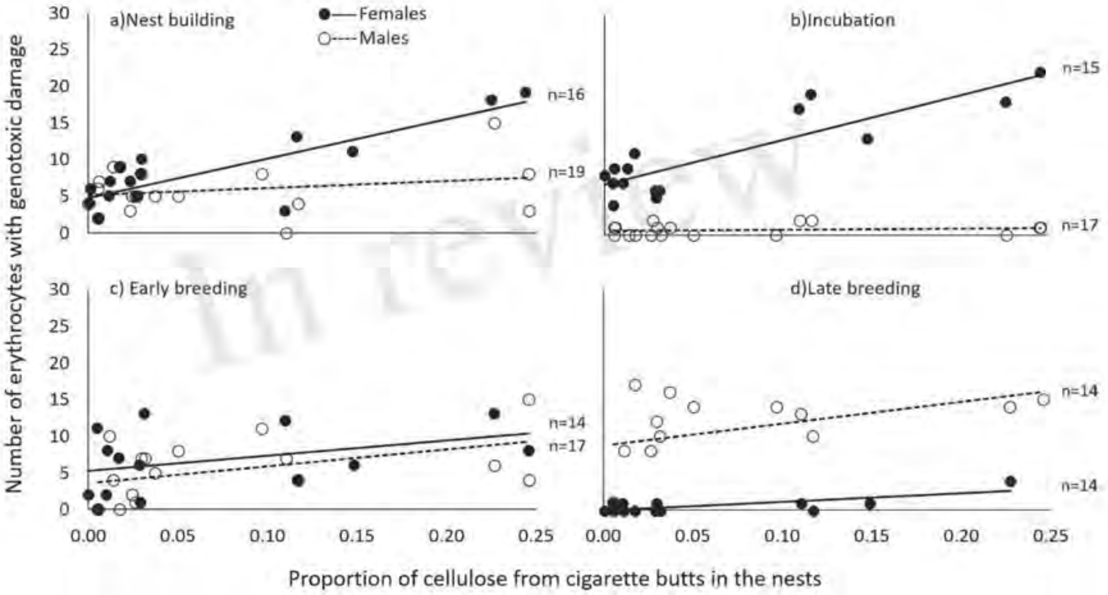

a)Nest building
●— Females
○---- Males
b)Incubation
c) Early breeding
d)Late breeding
n=16
n=19
n=15
n=17
n=14
n=17
n=14
n=14
Number of erythrocytes with genotoxic damage
0
5
10
15
20
25
30
0.00
0.05
0.10
0.15
0.20
0.25
Proportion of cellulose from cigarette butts in the nests

Figure 3.TIF

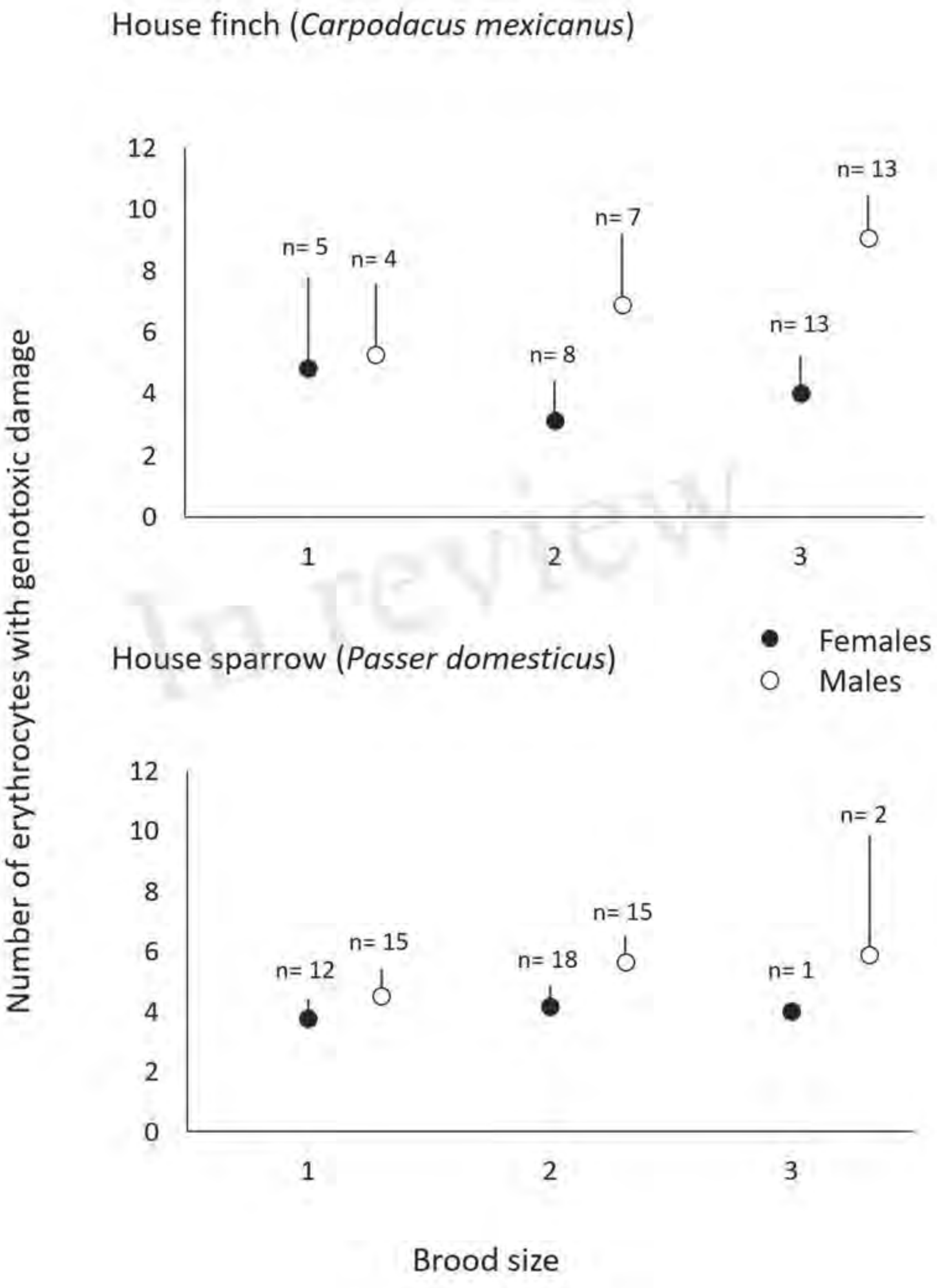
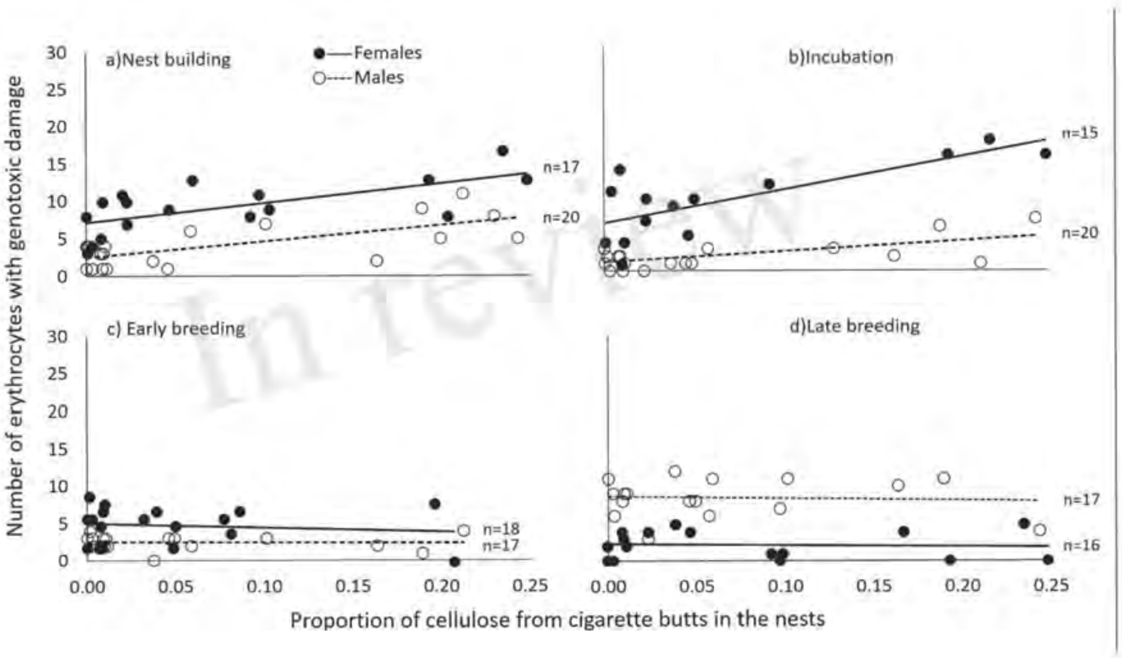

House finch (*Carpodacus mexicanus*)
12
10
8
6
4
2
0
n= 5
n= 4
n= 7
n= 13
n= 8
n= 13
1
2
3
● Females
○ Males
House sparrow (*Passer domesticus*)
12
10
8
6
4
2
0
n= 12
n= 15
n= 18
n= 15
n= 1
n= 2
1
2
3
Number of erythrocytes with genotoxic damage
Brood size

Figure 4.TIF


a)Nest building
b)Incubation
c) Early breeding
d)Late breeding
●—Females
○----Males
n=17
n=20
n=15
n=20
n=18
n=17
n=17
n=16
30
25
20
15
10
5
0
0.00
0.05
0.10
0.15
0.20
0.25
Number of erythrocytes with genotoxic damage
Proportion of cellulose from cigarette butts in the nests

V. CAPÍTULO IV. “An experimental demonstration that house finches add cigarette butts in response to ectoparasites”

Resumen

Un reto al que se enfrentan diferentes especies en los ambientes urbanos es la basura. Existen muchos estudios que han encontrado que la basura es peligrosa de muchas formas para la fauna silvestre. En la Ciudad de México encontramos filtros de cigarro como material de nido en algunas especies de aves. En nidos de gorrión inglés y de pinzón mexicano, los filtros están relacionados negativamente con la cantidad de ectoparásitos, pero también provocan daño genotóxico en los pollos y en los padres que los cuidan. Sin embargo, aún desconocemos por qué las aves agregan este material a sus nidos. En este trabajo, exploramos la hipótesis de que el pinzón mexicano (*Carpodacus mexicanus*) responde a la carga ectoparasitaria ajustando la cantidad de colillas que incorpora en sus nidos. Realizamos cambios de camas de nido por una artificial y los dividimos en tres tratamientos 1) ectoparásitos vivos (aumentado), 2) ectoparásitos muertos (control 1) y 3) simulación de la manipulación (control 2). Nuestra hipótesis fue parcialmente apoyada por nuestros datos. Las hembras agregaron más material de filtros al tratamiento de ectoparásitos vivos. Además, los individuos que pusieron más filtros en su cama original, también pusieron más en la cama artificial. Esto, podría deberse a experiencia previa o a experiencia temprana (plasticidad en la conducta durante el desarrollo). Si el uso de este material antiparasitario es algo que se originó en las ciudades, podría ser una causa adicional del por qué esta especie ha tenido mucho éxito en ambientes urbanos. Así, mostramos que es importante estudiar la relación de los animales con los “recursos sintéticos” que les ofrecen las ciudades y cómo han aprendido a utilizarlos.

Capítulo en revisión.

Journal of Avian Biology

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An experimental demonstration that house finches add cigarette butts in response to ectoparasites

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Keywords:	Self-medication, urban birds, nest materials
Abstract:	Urban species are expose to different types of resources in cities, like rubbish. There are many studies that have found that rubbish is dangerous in many ways to wildlife. In Mexico City we found that house finches use cigarette butts as nest material. In nests of house finches and house sparrows, material from cigarette butts are negatively related to the amount of ectoparasites. Also this material seems to affect genotoxic damage in chicks and parents However, we don´t know if there is a particular reason from which birds use cigarette butts to build their nests. In this work we tested the hypothesis that house finches (Carpodacus mexicanus) adjusts the amount of cigarette butts in response to ectoparasites load. We made a manipulation to their nests where we change the lining of their nests (getting rid of the cigarette butts and ectoparasites in that part of the nests) and then we randomly assigned each nests to a different treatment: 1) live ectoparasites (ectoparasites added), 2) dead ectoparasites (negative control) and 3) simulation of the manipulation (second negative control). Our hypothesis was supported by our data, females added more material from cigarette butts in the live ectoparasites treatment. Additionally, we found that females added more butts material when they had already used more butts in their first lining (before manipulation). Apparently the addition of this material is the result of several stimuli. Among these could be the experience previous the manipulation (that could include early experience) and the ectoparasite load.

An experimental demonstration that house finches add cigarette butts in response to ectoparasites

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Abstract

Urban species are expose to different types of resources in cities, like rubbish. There are many studies that have found that rubbish is dangerous in many ways to wildlife. In Mexico City we found that house finches use cigarette butts as nest material. In nests of house finches and house sparrows, material from cigarette butts are negatively related to the amount of ectoparasites. Also this material seems to affect genotoxic damage in chicks and parents However, we don´t know if there is a particular reason from which birds use cigarette butts to build their nests. In this work we tested the hypothesis that house finches (*Carpodacus mexicanus*) adjusts the amount of cigarette butts in response to ectoparasites load. We made a manipulation to their nests where we change the lining of their nests (getting rid of the cigarette butts and ectoparasites in that part of the nests) and then we randomly assigned each nests to a different treatment: 1) live ectoparasites (ectoparasites added), 2) dead ectoparasites (negative control) and 3) simulation of the manipulation (second negative control). Our hypothesis was supported by our data, females added more material from cigarette butts in the live ectoparasites treatment. Additionally, we found that females added more butts material when they had already used more butts in their first lining (before manipulation). Apparently the addition of this material is the result of several stimuli. Among these could be the experience previous the manipulation (that could include early experience) and the ectoparasite load.

Keywords. Self-medication, urban birds, nest materials

Introduction

Urban environments pose challenges that are often at odds with those historically experienced by species reaching the cities (Andersson 2006, Slabbekoorn and Den Boer-Visser 2006, Romieu et al. 2008, Geens et al. 2009, Dominoni and Partecke 2015). Consequently, urban ecologists frequently focus on studying the attributes that have allowed certain species to successfully colonise urban environments (Slabbekoorn and Den Boer-Visser 2006, Macías Garcia et al. 2016). One such trait is behavioural plasticity , which together with the expression of innovative behaviours (or of traditional behaviours translated to novel situations) have been found to be often associated with the colonisation of cities (Reader 2003, Antczak et al. 2010, Lowry et al. 2013). These allow the exploitation of new resources and helps adjusting the way in which species deal with their novel environment (Piaget 1978, Snell-Rood 2013, Sol et al. 2013). At least initially, urban species are able to exploit both human-provided and naturally-occurring resources (Mckinney 2002). In the cities, lack of feedback loops between the production and the use of such resources allow exploiter species to increase their abundance. Species that are unable to replace their natural resources with those found in cities (for instance because of lack of behavioural plasticity) do not become urban dwellers (Beissinger and Osborne 1982, Blair 2001, Mckinney 2006).

Some behavioural adjustments or innovations by birds have been extensively studied in the changing and challenging city environments. Examples include the use of food alternatives (Galbraith et al. 2015, Scott et al. 2015), and adjustments to song attributes such as minimum frequency (Slabbekoorn and Peet 2003, Burhans and Thompson 2006, Nemeth and Brumm 2009, Bermúdez-Cuamatzin et al. 2010), duration (Ríos-Chelén et al. 2013), and timing (Gil et al. 2014). Conversely, nest building behaviour has been thought to be evolutionarily conserved. Building a nest is an essential part of avian breeding and it entails making a series of decisions such as where

and what with to construct the nest, which depend on the environment they live on(Martin 1993, Collias 1997, Mazgajski 2007) . Those decisions in turn determine the structure, thermal properties, resistance and vulnerability of the nest (Nilsson 1984, Healy et al. 2008, Bailey et al. 2014) and sometimes also their chemical attributes (Clark and Mason 1988).

While some traditional nest materials and nesting sites are sufficiently abundant in cities, others are not, and must therefore be substituted with anthropogenic materials and structures which are locally available; this requires a degree of plasticity on the part of the breeding birds (Mennerat et al. 2009, Wang et al. 2009, Moreno et al. 2010). Thus, urban birds nest on buildings instead of on cliffs (Møller 2010) and use paper and other urban materials for nest building (Wang et al. 2009). Nesting innovations could be among the factors that allow some species adapt to cities, given that failing to use suitable urban nest sites and materials must impair reproduction (Reader and Laland 2003). There are some reports of anthropogenic materials finding their way into avian nests including plastics (Morris 1980, Hartwig et al. 2007, Antczak et al. 2010), paper (Wang et al. 2009) and cigarette butts (Igic et al. 2009). Interestingly, there are also reports of the use of novel nest materials in nature, as a consequence of the introduction of exotic species or of anthropogenic materials (Van Riper III 1977). In addition to availability, it is possible that unusual materials are used in nest building because they resemble traditional materials in some relevant property (Antczak et al. 2010), although they often have other attributes which have additional consequences for nesting birds(Suárez-Rodriguez et al. 2013, Suárez-Rodriguez and Macías Garcia 2014).

While most materials brought into the nest appear to have some clear structural or thermal function (e.g. (Hilton et al. 2004), others may perform a variety of functions, from mate attraction and pair bonding (Brouwer and Komdeur 2004, Veiga et al. 2006, Polo et al. 2010), to

promoting hatching (Rendell and Verbeek 1996, Dawson 2004, Polo et al. 2015) or fledging success (for instance by enhancing chick immune responses;(Gwinner et al. 2000, Mennerat et al. 2009). Some of these effects can be the consequence of a reduction in the probability or intensity of ectoparasitic infestation (e.g. Dawson 2004), as demonstrated in cases where green leaves with arthropod-repellent properties are brought to the nest (e.g. Clark 1991). Chick survival can also be promoted by adding materials that reduce the risk of nest predation with materials that conceal the nest/nestlings (i.e. provide camouflage; Møller 1987).

One favoured hypothesis to explain the often reported addition of green plant materials to bird nests I their potential role in deterring or even harming ectoparasites. This is because ectoparasites can lower the condition of parents and chicks, and may reduce the survival of the latter (Møller 1993, Lehmann 1993, Fitze et al. 2004). This constitutes a relentless selective pressure promoting the evolution of bird defences against ectoparasites (Bucher 1988, Hart 1992, Heeb et al. 1998, Cantarero et al. 2013). Studies on common starlings (*Sturnus vulgaris*; Clark & Mason 1988), blue tits (*Cyanistes caeruleus*; Lafuma, Lambrechts & Raymond 2001; Petit *et al.* 2002; Tomás *et al.* 2010) and Bonelli's eagle (*Aquila fasciata*; Ontiveros, Caro & Pleguezuelos 2007) have shown that the addition of particular green plant materials have negative effects on the amount of ectoparasites on nests. Green plant materials often contain secondary metabolic compounds likely evolved because they reduce the risk of herbivory by either killing or repelling arthropod herbivores (Wimberger 1984, Dubiec et al. 2013). By carrying those materials into their nests, birds can benefit from the effects of such substances on their arthropod ectoparasites (Clark and Mason 1988, Clark 1991, Dubiec et al. 2013). Whether green aromatic plant parts were initially brought into the nest because of their structural properties, or as a consequence of their scent and scent-linked attributes, the fact is that currently they are often taken into the nest

because they repel ectoparasites (Clark and Mason 1987, Petit et al. 2002, Gwinner and Berger 2008).

In 2011 we found that House finches (*Carpodacus mexicanus*) and House sparrows (*Passer domesticus*) in Mexico City add cigarette butts to their nests. We also found that the amount of this material is negatively related to the nest ectoparasite load (Suárez-Rodríguez et al. 2013). Some aspects of chick performance are affected by this toxic material (Suárez-Rodríguez and Macías Garcia 2014) and now we know that also the parents experience toxic costs from using cigarette butts (Suárez-Rodríguez et al. under review). The cellulose fibres that make up the cigarette butts seem to have the thermal and structural properties of other materials naturally used to line passerine nests, such as down and wool, but in addition, the election of this material may help to increase reproductive success by decreasing the effects of ectoparasites on chicks. However, we still ignore whether butts are brought into nests in response of the presence of ectoparasites, or whether they are taken indiscriminately to provide thermal nest lining. Here we addressed the possibility that the house finches from Mexico City add cigarette butts to their nests in response to the presence of ectoparasites in their nests.

Methods

We conducted an experiment that consisted on removing the lining from house finches nests shortly after the chicks hatched, and substituting it with an artificial, parasite- and cigarette butt-free lining. Parasites, either dead (control) or alive were then added, or mock added, and the nest -including the new lining was- collected after the chicks fledged. We counted the number of parasites and of cigarette-butt fibres in the original lining and in the artificial lining, and evaluated the effect of the treatment on the addition of butts after the manipulation.

Study site

We studied a house finch population at the main campus of the Universidad Nacional Autónoma de México (UNAM) within Mexico City. We observed nests/breeding pairs every other day from January 2015, and waited for pairs to finish building and egg laying. Birds were identified as they had colour and metal rings fitted during the previous season (Suárez-Rodriguez et al., submitted). We have observed nest sanitation, including bringing new nest materials, throughout the breeding season. This afforded the possibility of manipulating nest parasite content and then recording whether and by how much the amount of cigarette butt fibres was increased in response to the manipulation. At hatching (±1 day) we swapped the lining of the nests with a new artificial one (see below). Only the lining was changed, and only after hatching, because house finches from this population usually abandon nests when they perceive unusual changes in the nest before the eggs hatch (pers obs).

Design of artificial lining

We made artificial nest linings (AL) with circular pieces of brown felt to substitute the original lining, which was normally made of feathers, cotton, hair and butts seemingly assembled to provide a soft, thermally-insulating bed (see also temperature analysis). A wreath of approximately 0.05 g of dry thin branches of Glossy Privet shrub (*Ligustrum lucidum*) was woven with ≈0.03 g of Kikuyu grass (*Pennisetum clandestinum*) leaves to form a small cup into which the felt piece was fixed to create the new lining bed. We have found both plants in house finch nests. Since the grass depends largely on silicification to fend off herbivores, and as we did not use the tissues (e.g. leaves and flowers) of the privet shrub that concentrate its chemical defences, our choice of materials did not influence the results (also, all treatments received AL build in the same manner; see below). The artificial linings had the shape and measures of natural linings (≈8 cm of diameter and ≈2 cm deep) and the nest owners were able to add materials to it (. To test whether

our manipulation altered the temperature of the nest (which could influence ectoparasite load; Hubálek, Halouzka & Juricova 2003; Proctor 2003) we measured nest temperature two days before and two days after the lining change, between 10 am and 1 pm, with an infrared thermometer (Extech™42529; ± 0.05 °C).

Collection of ticks and manipulation

To decide how many parasites to add in our treatments, we collected five nest linings prior to the experiment, and used the average number of ectoparasites found in them (70 ± 10.7) to increase the ectoparasite load. The same ectoparasites extracted from the first linings (ticks of the genus *Haemolaelaps*) were used in the subsequent manipulation; ticks were recognised by the presence of a hypostome (blood-sucking structure). Ticks were collected in 50 ml Eppendorf tubes using a Berlese-Tullgren funnel, then they were isolated with a brush under a stereoscopic microscope (Carl Zeiss Stemi), and separated in Eppendorf vials containing 70 ticks each, to use as stimuli. Ticks used in the dead (DE) treatment were immersed in 70 % alcohol for 24 hours and allowed to dry for another 24 hours.

After swapping the original (FL) with the artificial (AL) lining, we randomly assigned each nest to one of three different treatments: 1) live ectoparasites (LE), 2) dead ectoparasites (DE) and 3) no ectoparasites (NE). We placed the live mites within 48 hours after their capture. In the NE treatment we simulated adding the ectoparasites with an empty Eppendorf.

Collection of nests

The first (FL) and the artificial (AL) linings were cleaned under a Berlese-Tullgren funnel as in (Suárez-Rodríguez et al. 2013, Suárez-Rodríguez and Macías Garcia 2014). In each case we took apart the constitutive materials and weighted them. Thus we obtained a measure of the amount

of cigarette butts that the birds had put into their nests before (FL) and after the manipulation (AL).

Statistical analysis

Nest temperature influences ectoparasite load, and may also determine the use of cigarette butts if these are added for thermal reasons. To explore whether our treatment affected the temperature we applied a paired *t*-test to compare the temperature before and after the manipulation. Additionally, we applied an ANOVA to compare nest temperatures among treatments, expecting no effect of the change of lining or of treatment.

To examine variation in the amount of cigarette butts that birds added to the artificial lining we used normal lineal model analysis. The independent variable was the amount of cigarette butts in the artificial lining (taken to be a response of the treatments LE, DE and NE; independent factor). Since an initial assessment of the nest/nest site may determine the subsequent addition of cigarette butts by the parents, we used as covariable the amount of cigarette butts in the first lining. Also, if butt cotton fibres are added for thermal reasons, variation in temperature among nests could determine how many more are added by the parents, thus temperature in the two days following the manipulation was also added as a covariable. We employed the Box-Cox transformation in the response variable (Box and Cox 1964).

We tested different hypotheses (competing models) based on the different combinations of variables. These were models with only one variable: 1) cigarette butts in the first lining (FL), 2) temperature and 3) treatment. We also proved two-way interaction models, but only additive hypotheses with three variables. We compared the fit of the models using the corrected version for small samples of the Akaike Information Criterion (AICc). The lowest value of the AICc indicates

the best-fitting model and a difference between two models in AICc values (ΔAICc) larger than two indicates a clear difference in their fit to the data (Burnham and Anderson 2002).

Results

The population that we study in Mexico City has shown to be very sensitive to manipulations, so we managed to conduct our experiment in natural conditions. Furthermore, we tried not to disturb them before egg laying, when they are less likely to abandon their nests. We took advantage of the maintenance behaviour that this species present to modify the structure and the ectoparasite load of the nests. Even when we just changed the lining of the nest after hatchling, the adjustment of materials was evident and significant.

We didn´t find significant differences in temperature before and after the changing of the lining (Paired t_{31} = -0.33, P=0.7437). We also didn´t found temperature differences among treatments ($F_{2,29}$=1.591, P=0.221). So we successfully controlled the change in temperature by randomizing the nests to the different treatments and by using the felt for lining.

At the end of the experiment we could recover 10 nests of live ectoparasites, 10 nests of dead ectoparasites and 12 of the non ectoparasites added treatment. Three models were supported by our data (ΔAIC < 2). In this models, the treatment and the cigarette butts from the first lining (FL) were important to explain the number of butts that females incorporated to the artificial lining (Table 1). The Box-Cox transformation indicated us to use $1/(y^2)$ from a λ=-2 and a log likelihood= 27.9 (Note that since the transformation is the inverse the interpretation we made is inverse too). Three models were equally supported by our data. The firs one (lowest AICc value) showed that the amount of cigarette butts that female added in the artificial lining were related to the treatment, the no ectoparasite treatment (NE) had slightly more butt material (β=-0.03±0.07) than the dead ectoparasite (DE; in the intercept) treatment but the live ectoparasites treatment

(LE) had more butt material (β=-0.21±0.07; Figure 1; Table 2). The second model has treatment as an important factor to explain the butts added, here the tendencies of the previous model are maintained (NE: β=-0.07±-0.073; LE: β=-0.07±-2.61). In addition, this model has the variable of the amount of butts in their original lining (FL: β=-0.50±-1.27.53; Table 2 and Figure 2). The last supported model also showed that females that had more butts in their first lining also added more butts to the artificial linings (β=-1.02±0.48; Figure 2; Table 2).

Discussion

The use of novel nest materials may be very convenient for species that live within cities. If such materials have particular attributes that promote the birds' fitness, then their use would be favoured by selection. Cigarette butts provide a novel tool to fight against parasitism during chick development and, for adults, while incubating. This benefits, however, need not be related to the cues used by birds to collect and add such materials to their nests. For example, if cigarette butts are perceived as similar to fur or down, birds might take them to the nests as thermally-insulating nest lining, and their effect in reducing ectoparasite load would be a collateral consequence. Yet since cigarette butts are toxic, their use as substitutes of fur or down should be penalised unless the latter were scarce, or if the additional benefit of repelling ectoparasites did not overcompensates their toxic effects. Butts are found in most sections of house finch nests, but most are weaved into the lining.

Our hypothesis that house finches bring fibres from discarded butts into their nests in response to the presence of ectoparasites was supported by our data. Additionally, we found that in nests where birds had initially laid more fibres, they also added more following the bed lining manipulation. It is possible that the latter reflects the need in certain nests to improve thermal insulation by adding fur-like material, yet since temperature was not included in the best

supported models, we believe that this was not the case. Instead, it is possible that the initial addition of butt material was a response to the parasite load experienced by the females during nest building, which would accord with the fact that having live ticks added to their nests did promote the addition of fibres from cigarette butts.

In different types of environments, populations of the same species use different types of materials and sites for nesting, as long as they share particular attributes (Mennerat et al. 2009). We do not have information of house finches in more natural populations using plant material that reduce the ectoparasite load in their nests. In Mexico City, house finches have access to wild tobacco plants (*Nicotiana glauca* and *N. tabacum*). However, we have not found these plants or other fresh green materials in their nests. It may be that *N. glauca* is not as potent as cigarette butts to repel arthropods, or that its presence in the nests is inconvenient for other reasons. Whether or not in natural environments house finches use nest materials to reduce ectoparasite load -of which we have no evidence-, the prevalence of this behaviour in the very large population at Mexico City, and its recent discovery at other cities (e.g. Ciudad Juarez; I. Delgadillo Ramirez pers. com.) suggests that plasticity in the use of (useful) nest materials may provide an extra reason of why this species has been so successful in colonising cities.

Females find, manipulate and collect the fibres form discarded cigarette butts, but it is not clear what cues are they using to find them. We know that house finches have the ability to discriminate odours (Amo et al. 2012, 2015), so they could use scent to home into the cigarette butts if such odour is naturally attractive to them. Additionally, they might learn to associate butt odour to the relief from ectoparasites cues; an association that could also be established while the chicks are in the nest, and influence their choice of nest materials when adults. These are not

mutually exclusive explanations, but the latter (learnt association between odour and comfort) could promote the rapid spread of this behaviour in the cities.

It is a matter of concern that cigarette butts, being toxic, are part of house finch nests in Mexico City. We have observed a breeding colony since 2011, and we have not come across evidence of long-term costs of using this material (Suárez-Rodríguez and Macías Garcia 2014). Yet a systematic evaluation of the demography and physiology of this population is necessary to determine whether the use of cigarette butts as nest materials is a positive or a detrimental novelty of these urban birds. What our experimental manipulations shows, nevertheless, is that cigarette butts are added in response to an increase in the number of ectoparasites in the nest, and thus must be regarded as a form of self-medication (Clayton and Wolfe 1993).

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Table 1. Models that examine variation in the amount of cigarette butts in the artificial lining (AL).

Models	Number of parameters (k)	N	AICc	ΔAICc
Treatment	3	32	-20.79	0.00
Treatment + FL	4	32	-20.07	0.72
FL	2	32	-20.01	0.78
Treatment + temperature	4	32	-18.38	2.41
FL + temperature	3	32	-18.13	2.66
Treatment + FL + temperature	5	32	-17.58	3.21
Null	1	32	-15.70	5.08
Temperature	6	32	-14.75	6.04
Treatment * temperature	2	32	-13.68	7.10

Table 2. Estimates of the three models that explain the addition of butts to the nests after treatments.

		AL~ Treatment	
	Estimate	SE	*P*
Intercept	0.99	0.05	19.66
Treatment: LE	-0.21	0.07	-2.96
Treatment: NE	-0.03	0.07	-0.45

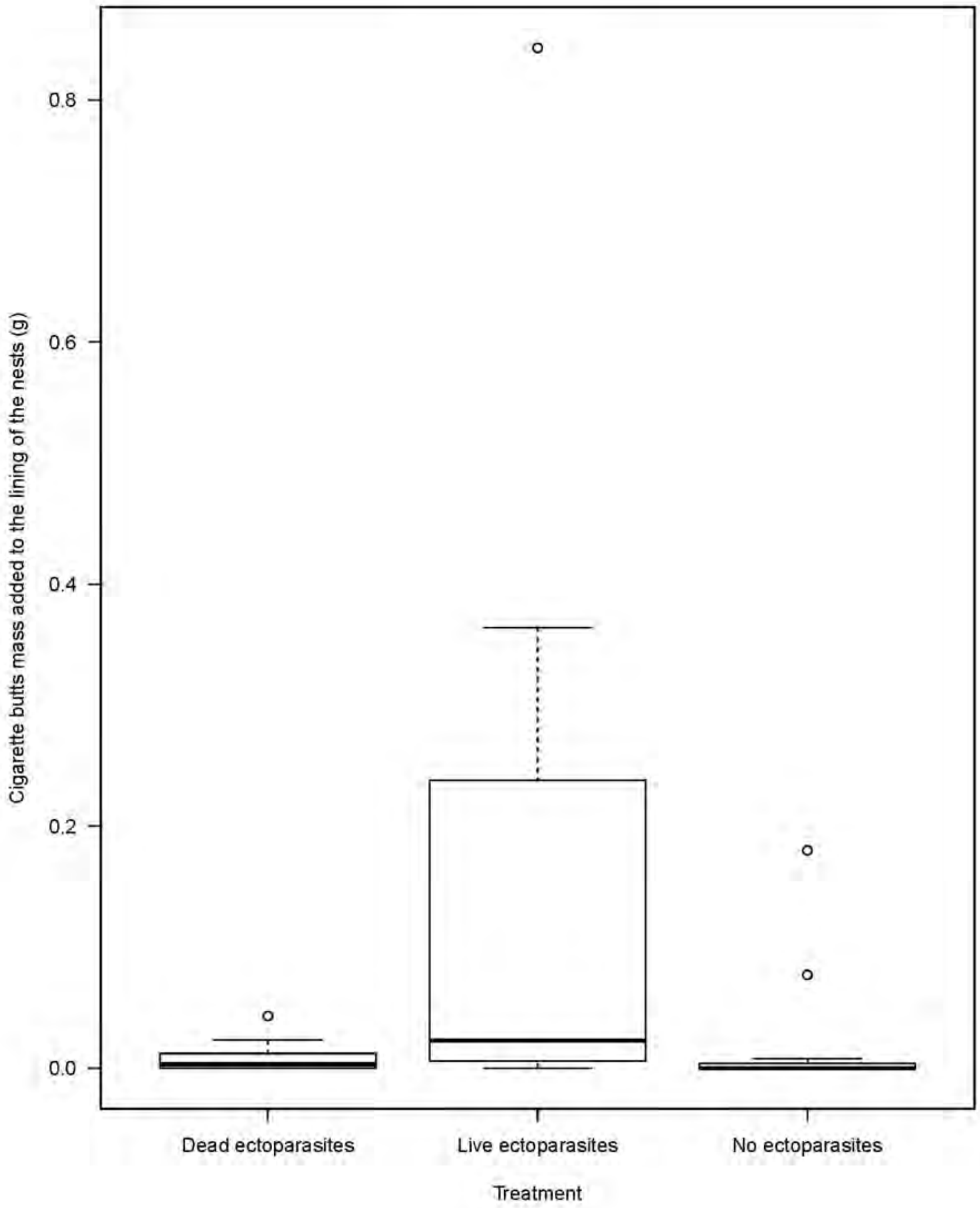
		AL~ Treatment + FL	
	Estimate	SE	P
(Intercept)	0.06	18.49	<0.001
Treatment: LE	0.07	-2.61	0.01
Treatment: NE	0.07	-0.73	0.47
FL	0.50	-1.27	0.21

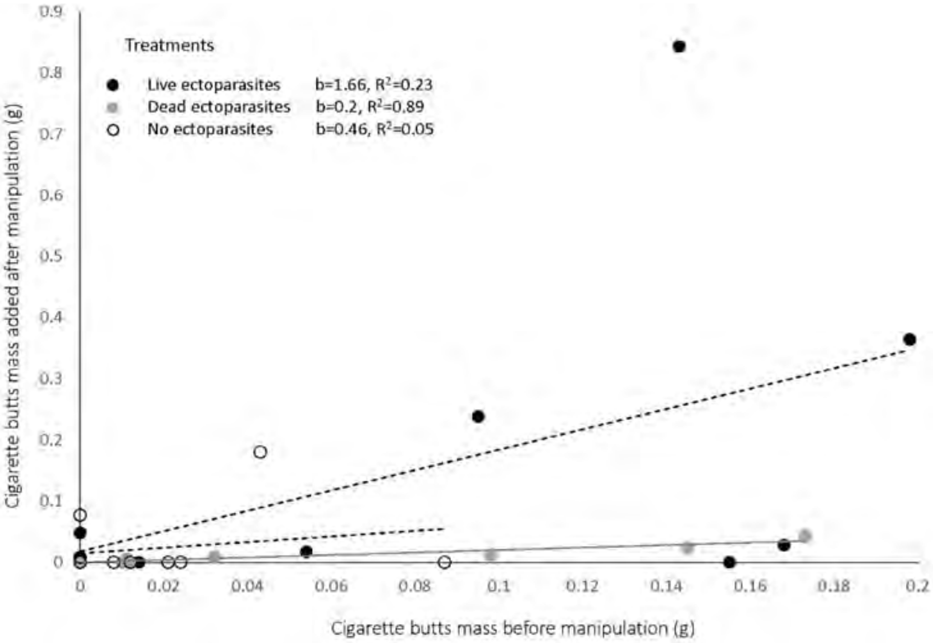
		AL~ FL	
	Estimate	SE	P
(Intercept)	0.96	0.04	25.56
FL	-1.02	0.48	-2.12

Figure legends

Figure 1. Box-plot of mass of butts added by female house finches to the lining of their nests following the treatments (see text).

Figure 2. Association between the initial amount (mass) of cigarette butts placed in the nest lining before manipulation and the amount added after the treatments (see text). (Non-significant regressions are indicated by dotted lines; b= slope).


Cigarette butts mass added to the lining of the nests (g)
0.0
0.2
0.4
0.6
0.8
Dead ectoparasites
Live ectoparasites
No ectoparasites
Treatment


Treatments
● Live ectoparasites b=1.66, R²=0.23
● Dead ectoparasites b=0.2, R²=0.89
○ No ectoparasites b=0.46, R²=0.05
Cigarette butts mass added after manipulation (g)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Cigarette butts mass before manipulation (g)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2

VI. DISCUSIÓN GENERAL

6.1 Objetivos logrados e hipótesis generadas

En el segundo capítulo de esta tesis nos interesaba explorar las consecuencias del uso de los filtros de cigarro en los huevos y pollos que crecen en el nido. El éxito de eclosión se vio afectado positivamente por los filtros, probablemente porque la presencia de parásitos incomoda a las hembras y reduce su eficiencia durante la incubación al obligarlas a acicalarse constantemente. Esto no afecta a los machos de *Carpodacus mexicanus* puesto que no incuban.

Otra ventaja importante después de la eclosión, es que los pollos pueden aprovechar mejor los nutrientes que obtienen del alimento, ya que las colillas de cigarro disminuyen la cantidad de ectoparásitos en el nido. Esto podría explicar que la ganancia en peso sea mayor en nidos con muchas colillas. La masa de los pollos es un predictor de éxito de supervivencia, como también lo es la talla al emplumado. No encontramos una relación de la cantidad de colillas con la tasa de crecimiento en masa. Una posible explicación es que la ganancia de masa tiene menos restricciones ontogenéticas que la talla (Gould 1966), pero esto es algo que no sabemos con certeza. Sin embargo, proponemos que la condición de los pollos, debida a una mayor ganancia de peso, es una ventaja derivada del uso de filtros de cigarro en la construcción de los nidos. Sería interesante verificar si esta aparente ventaja repercute en el éxito de reclutamiento.

El cigarro es tóxico y esperábamos encontrar evidencia de toxicidad en las aves que se exponen a las colillas fumadas. Sin embargo, no encontramos efectos negativos directos en los pollos. La genotoxicidad que detectamos puede o no derivar en resultados perjudiciales a largo plazo. Si las mutaciones que son provocadas por las sustancias del cigarro se pasan de célula en célula o dañan sitios clave en los genes (como promotores) podría existir mutagenicidad que podría derivar en efectos graves como procesos cancerígenos. Además, es importante considerar que las células germinales son especialmente importantes, ya que podrían producir mutaciones en la descendencia y esto provocaría efectos en el éxito reproductivo. Sin embargo, con el método que utilizamos no podemos saberlo, pero es importante tenerlo en cuenta en futuras investigaciones.

Sabemos que en las aves las células de la sangre se filtra en ciclos de aproximadamente 30 días (Rodnan et al. 1956), aunque sabemos que puede detectarse antes de este tiempo (ver capítulo dos). Eventualmente la sangre de las aves se recicla en su totalidad y esto podría permitir que no tengan efectos negativos más graves por la toxicidad de los filtros de cigarro. Para determinar si el daño genotóxico observado conduce a mutagénesis sería preciso realizar un estudio a largo plazo. En ausencia de mutagénesis, podría ser que al dejar el nido y estar aproximadamente seis meses sin contacto con colillas tanto pollos como adultos podrían detoxificarse por completo y no sufrir consecuencias duraderas. Dado que medimos toxicidad en una línea celular de vida relativamente corta, podríamos estar subestimando el daño inducido por la exposición a las substancias de las colillas, de manera que sería prudente evaluar el daño indicado por

sustancias en las colillas, en tejidos de más larga vida, como por ejemplo el eritropoyético en la médula espinal. Evaluar la respuesta a la intoxicación por colillas de cigarro usando técnicas genómicas nos daría a la vez información sobre los mecanismos de detoxificación, y sobre procesos de adaptación de las aves a ambientes tóxicos como lo son las ciudades.

En el capítulo tres realizamos una evaluación de los adultos que cuidan a los huevos y pollos. Debido a que obtuvimos una muestra relativamente grande de ambas especies, *Passer domesticus* y *Carpodacus mexicanus*, aprovechamos la oportunidad de explorar los efectos genotóxicos bajo dos contextos diferentes de cuidado parental.

La genotoxicidad que detectamos se relacionó con las diferencias conductuales entre machos y hembras asociadas a los roles parentales. Aparentemente, las propiedades de los materiales del nido pueden afectar de manera diferencial a machos y a hembras. Esto provocaría que los costos por el cuidado parental tuvieran un peso mayor para las hembras de *C. mexicanus*, ya que ellas se encargan por completo de las primeras dos etapas de la anidación (construcción del nido y la incubación). En *P. domesticus* el costo de agregar filtros al nido parece ser compartido de manera casi igual entre machos y hembras. Si la genotoxicidad reflejara un costo a largo plazo, tal vez este efecto podría representar un costo importante para el cuidado monoparental del pinzón mexicano. No obstante requerimos de un estudio más detallado para conocer de qué forma y qué tanto impacto tiene en las hembras esta genotoxicidad.

Finalmente, en el tercer capítulo nos preguntamos cuál es el estímulo que impulsa a estas especies a colectar y agregar filtros de cigarro a las camas de sus nidos.

Manipulando la cantidad de ectoparásitos vivos y muertos (control), encontramos que la cantidad de colillas agregadas después del tratamiento está explicada por 1) el haber percibido un aumento (experimental) en la cantidad de ectoparásitos, y 2) la cantidad de colillas que habían puesto originalmente en el nido (que podría también haber ocurrido en respuesta a l número inicial de parásitos). Las hembras de pinzón mexicano agregaron más material de filtros a los nidos con más ectoparásitos vivos. Aparentemente la elección de este material es una combinación de la posible experiencia previa (al inicio del evento de anidación) y un ajuste de las condiciones actuales (adición de parásitos). Así las hembras pueden evaluar el estado de su nido constantemente y manipular su contenido durante la anidación.

6.2 Direcciones futuras

7.2.1 Efectos en la adecuación (Capítulos 1 y 2)

Este trabajo da paso a distintas líneas de investigación en el tema de los materiales de nido y su efecto en el mismo y en los individuos que tienen interacción con él. El tema de los materiales de nido ha sido poco estudiado en comparación con otras conductas, como el canto, de las aves. Sin embargo, es una estructura muy importante, ya que los pollos pasan mucho tiempo en este microambiente. Necesitamos seguir indagando sobre los efectos negativos y positivos que encontramos para conocer a más largo plazo si los efectos se mantienen, qué otros efectos tienen y si las aves aprenden de estas experiencias.

Ahora que sabemos que las hembras de *C. mexicanus* podrían hacer más eficiente la incubación debido a la disminución de ectoparásitos, es importante probar esta hipótesis de manera más directa. Se podría iniciar con observaciones de conducta de acicalamiento. Por ejemplo, se podría cuantificar acicalamiento en nidos con y sin filtros de cigarro. Esto nos permitiría saber si las hembras de nidos sin filtros salen más del nido y/o se acicalan más durante la incubación. Posteriormente podríamos comparar el éxito de eclosión de ambos escenarios. Para manipular la cantidad de filtros podríamos hacer uso de experimentos en cautiverio, o utilizar la variación natural (en vida libre) de la cantidad de filtros que agregan las hembras a los nidos.

La genotoxicidad podría tener implicaciones muy importantes en las aves. En estos años de trabajo con *C. mexicanus* y *P. domesticus* no hemos observado alguna señal somática de procesos cancerígenos en los individuos, pero no hicimos una búsqueda sistemática. También pensamos que es importante realizar un estudio demográfico, para saber si la exposición a colillas puede afectar medidas directas de adecuación como la supervivencia después de volantones. La mayoría de los pollos se reproducen en la siguiente primavera, pero los efectos fisiológicos de la exposición a colillas podrían retrasar su reproducción, reducir su fertilidad o afectar a los descendientes y su supervivencia.

7.2.2 Más allá de los ectoparásitos

No sabemos cómo es la plasticidad de la construcción del nido; más específicamente la elección de materiales, entre grupos taxonómicos. Sabemos que la diversidad más alta de

nidos ocurre en aves altriciales paseriformes (Starck and Ricklefs 1998, Macías Garcia et al. 2016). Hasta donde sabemos todas las aves experimentan la presión selectiva que imponen los ectoparásitos. Sin embargo, no sabemos si hay la plasticidad en uso de sitios y materiales de nido tiene un componente filogenético. Existen estudios que demuestran que la complejidad del canto está asociada con la pertenencia a clados particulares (e.g. Paseriformes Oscinos y Suboscinos, o no Paseriformes; Feenders et al. 2008). Además, conocemos las relaciones filogenéticas de esta característica de las aves (Feenders et al. 2008), pero no existen estudios similares sobre el componente filogenético en la construcción del nido. El conjuntar ambos rasgos en un análisis comparativo permitiría evaluar si el hecho de que las aves paseriformes muestren un área de aprendizaje muy desarrollada, que les permite ajustar y aumentar el repertorio de sus cantos, se relaciona con algún aspecto de la construcción del nido (como la plasticidad en el uso de materiales, o en la elección de sustratos), debido a que el aprendizaje es muy importante en la construcción e incorporación de nuevos materiales. Además de que el movimiento motor fino, que se podría requerir para tejer el nido, está estrechamente relacionado con los núcleos de canto.

Debido al efecto de la nicotina (y otras sustancias del cigarro) en el organismo, de muchas especies, ha provocado una serie de investigaciones sobre el tema. Es interesante saber que los efectos que se han encontrado en modelos animales, como la rata, se mantienen en los humanos. Algunos de estos describen el proceso de adicción y el desarrollo de genotoxicidad. No obstante, al ser un tipo de basura que tiene tanto impacto directo en la salud de las personas, también debemos pensar en lo que pasa con otro tipo de animales. Shingo y Kito (2005), encontraron que inyectar nicotina a ratas de laboratorio disminuye la neurogénesis en la zona del hipocampo. Esto podría ser de gran interés en estas aves paseriformes, ya que el inicio de la época reproductiva induce la neurogénesis de los centros vocales de los machos para la producción del canto (Alvarez-Buylla and Theelen 1988, Alvarez-Buylla and Ling 1992, Hofman et al. 2002). El utilizar y manipular los filtros podría traer consecuencias a los machos que dependen del desarrollo del canto para atraer pareja. Esta idea podría ser explorada para saber si no ocurre o podría ocurrir en los ambientes urbanos, ya que podría representar una amenaza para el éxito reproductivo de las aves.

Por otra parte, se ha discutido mucho sobre los beneficios de la plasticidad de la conducta (Dingemanse et al. 2010, 2012, Ghalambor et al. 2013, Grunst et al. 2014). Por distintos trabajos, sabemos que la temeridad y la innovación, a veces, aumentan la probabilidad de permanencia de una especie en ambientes novedosos. Probablemente, resulte mejor utilizar un material desconocido, pero parecido al natural que podría igualar o aumentar el efecto en los nidos. Por ejemplo, los filtros de cigarro son un material que está disponible y es muy abundante en donde *P. domesticus* y *C. mexicanus* habitan. Este material podría hacer que estas aves ahorren energía al estar cerca. No sabemos si su uso inició como una necesidad estructural o de propiedades físicas del nido. Sin embargo, sus propiedades químicas parecen tener efectos importantes en la vida de las aves. Es por estas razones que es importante hacer un seguimiento de esta conducta y de cómo afecta la salud de las aves. Estudios a largo plazo, en vida libre o en cautiverio, nos podrían ayudar a determinar si los filtros de cigarro realmente están afectando a las poblaciones

que estudiamos. Además, con este trabajo invitamos a la investigación de los efectos de los recursos artificiales que muchas especies utilizan en los ambientes urbanos. Necesitamos explorar sus efectos positivos y negativos en las poblaciones y así fomentar una conciencia sobre el daño que hacen las actividades humanas, y lo que producen, a las especies que quedan atrapadas en las ciudades.

VII. REFERENCIAS

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Table 2.1. Values of β used to calculate of the relative contributions of the different levels of factor sex (males, females) and breeding stage (nest building, incubation, early and late breeding) to the overall model. Slope = the steepness of the association between amount of cigarette butts and the number of cells with genotoxic damage per 2000.

Breeding moment	Intercept				Slope			
	Females	β used	Males	β used	Females	β used	Males	β used
Nest building	1.656	β0, β2	1.652	β0	5.241*	β1, β6	1.523	β1
Incubation	1.983*	β0, β2, β3,10	-0.501	β0, β3,	4.657*	β1, β7, β13	1.842	β1, β7
Early breeding	1.668	β0, β2, β4, β11	1.347	β0, β4	2.788	β1, β8, β14	3.666*	β1, β8
Late breeding	-1.563	β0, β2, β5, β12	2.189*	β0, β5	12.335*	β1, β59, β15	2.454	β1, β59

Table S3. Five models evaluated to explain the amount of genotoxic damage in erythrocytes of *Carpodacus mexicanus* (house finch) males in the last two breeding stages (nest building, incubation, early and late breeding). We selected as the best model that with the smallest value of AIC.

Models	AIC	ΔAIC
Sex x brood size	**386.199**	**0.000**
Sex + brood size	387.186	0.987
Sex	388.150	1.951
Brood size	418.804	32.605
Null	420.325	34.126

Table S4. Five models evaluated to explain the amount of genotoxic damage in erythrocytes of *Passer domesticus* (house sparrow) males in the last two breeding stages (nest building, incubation, early and late breeding). We selected as the best model that with the smallest value of AIC.

Models	AIC	ΔAIC
Amount of cigarette butts x sex x breeding stage	**635.542**	**0.000**
Breeding stage x sex	674.539	38.997
Amount of cigarette butts x breeding stage + sex	801.355	165.813
Amount of cigarette butts + sex + breeding stage	826.121	190.579
Amount of cigarette butts x sex + breeding stage	828.098	192.556

Table S5. Description of the best model to explain the amount of genotoxic damage in erythrocytes of *Passer domesticus* (house sparrow). βn indicate the coefficients used to calculate the slopes and intercepts of particular factor levels (i.e. β2 quantifies the global contribution of females to the model; see below for an explanation on how we calculated the magnitude of the interaction effects).

B	Variable	Coefficient	SE	Z	*P*
β0	Intercept	0.954	0.175	5.455	< 0.001
β1	Amount of cigarette butts	4.709	1.145	4.113	< 0.001
β2	Sex: females	1.043	0.211	4.944	< 0.001
β3	Incubation	-0.683	0.295	-2.319	0.020
β4	Early breeding	-0.036	0.263	-0.136	0.892
β5	Late breeding	1.194	0.208	5.734	< 0.001
β6	Amount of cigarette butts x sex: females	-2.013	1.448	-1.391	0.164
β7	Amount of cigarette butts x breeding stage: incubation	0.906	2.059	0.440	0.660
β8	Amount of cigarette butts x breeding stage: early breeding	-4.970	2.565	-1.938	0.053
β9	Amount of cigarette butts x breeding stage: late breeding	-5.003	1.663	-3.008	0.003
β10	Sex: females x breeding stage: incubation	0.687	0.339	2.028	0.043
β11	Sex: females x breeding stage: early breeding	-0.273	0.316	-0.865	0.387
β12	Sex: females x breeding stage: late breeding	-2.348	0.331	-7.103	< 0.001
β13	Amount of cigarette butts x sex: females x breeding stage: incubation	0.408	2.412	0.169	0.866
β14	Amount of cigarette butts x sex: females x breeding stage: early breeding	1.214	3.121	0.389	0.697
β15	Amount of cigarette butts x sex: females x breeding stage: late breeding	1.497	2.831	0.529	0.597

Table S5.1. Values of β used to calculate of the relative contributions of the different levels of factor sex (males, females) and breeding stage (nest building, incubation, early and late breeding) to the overall model. Slope = the steepness of the association between amount of cigarette butts and the number of cells with genotoxic damage per 2000.

Breeding moment	Intercept				Slope			
	Females	B used	Males	B used	Females	B used	Males	B used
Nest building	1.997*	β0, β2	0.954	β0	2.696	β1, β6	4.709	β1
Incubation	2.001*	β0, β2, β3,10	0.271	β0, β3,	4.009	β1, β7, β13	5.614	β1, β7
Early breeding	1.688*	β0, β2, β4, β11	0.918	β0, β4	-1.060	β1, β8, β14	-0.261	β1, β8
Late breeding	0.844	β0, β2, β5, β12	2.148*	β0, β5	-0.811	β1, β59, β15	-0.294	β1, β59

Table S6. Five models evaluated to explain the amount of genotoxic damage in erythrocytes of *Passer domesticus* (house sparrow) males in the last two breeding stages (nest building, incubation, early and late breeding). We selected as the best model that with the smallest value of AIC.

Models	AIC	ΔAIC
Sex + brood size	**342.5974**	**0.000**
Brood size	346.103	3.506
Sex x brood size	346.3325	3.735
Sex	350.2487	7.651
Null	354.8781	12.281

Table S7. Mean (± standard deviation) frequency of erythrocytes with nuclear abnormalities/2000 cells, taken as an index of genotoxic damage, during four breeding stages (males + females) of *C. mexicanus* and *P. domesticus*.

C. mexicanus		
Breeding stage	Average	SD
Nest building	6.86	4.32
Incubation	5.41	6.39
Early breeding	5.93	4.3
Late breeding	5.75	6.37
P. domesticus		
Breeding stage	Average	SD
Nest building	6.51	4.14
Incubation	5.63	5.56
Early breeding	3.83	2.36
Late breeding	5.39	3.88

Table S8. To test if our assumptions of independence in the data were true, we applied a GLM (Poisson distributions of dependent variable) with only one individual, male or female, and one nest per breeding stage. We chose with random numbers in Excel each individual from our data base. We did this for both species. Five models evaluated to explain the amount of genotoxic damage in erythrocytes of *Carpodacus mexicanus* (house finch) among breeding stages (nest building, incubation, early and late breeding). We selected as the best model that with the smallest value of AIC.

Models	AIC	ΔAIC
Amount of cigarette butts x sex x breeding stage	**135.5286**	**0**
Amount of cigarette butts + sex x breeding stage	**137.2181**	**1.6895**
Sex x breeding stage	**137.3512**	**1.8226**
Amount of cigarette butts x sex	198.5734	63.0448
Amount of cigarette butts x sex + breeding stage	203.2028	67.6742

Table S9. Description of the best model to explain the amount of genotoxic damage in erythrocytes of *C. mexicanus*. This analysis was made from only one individual per nest per sex (N= 26; nest building n= 3 males, 4 females; incubation n= 3 males, 4 females; early breeding n= 4 males, 2 females; late breeding n= 3 males, 3 females).

Variable	Coefficient	SE	Z	*P*
Intercept	1.770	0.361	4.902	< 0.001
Amount of cigarette butts	2.723	5.841	0.466	0.641
Sex: females	-0.841	0.565	-1.487	0.137
Incubation	-1.685	0.911	-1.849	0.064
Early breeding	0.096	0.443	0.217	0.828
Late breeding	0.751	0.415	1.808	0.071
Amount of cigarette butts x sex: females	5.469	6.951	0.787	0.431
Amount of cigarette butts x breeding stage: incubation	-3.540	8.414	-0.421	0.674
Amount of cigarette butts x breeding stage: early breeding	-3.195	6.278	-0.509	0.611
Amount of cigarette butts x breeding stage: late breeding	-2.426	5.854	-0.415	0.679
Sex: females x breeding stage: incubation	2.818	1.031	2.734	0.006
Sex: females x breeding stage: early breeding	1.229	0.879	1.399	0.162
Sex: females x breeding stage: late breeding	-2.329	1.305	-1.784	0.074
Amount of cigarette butts x sex: females x breeding stage: incubation	-0.470	9.300	-0.051	0.960
Amount of cigarette butts x sex: females x breeding stage: early breeding	-20.979	30.884	-0.679	0.497
Amount of cigarette butts x sex: females x breeding stage: late breeding	-6.365	7.074	-0.900	0.368

Table S10. Five models evaluated to explain the amount of genotoxic damage in erythrocytes of *Passer domesticus* (house finch) among breeding stages (nest building, incubation, early and late breeding). We selected as the best model that with the smallest value of AIC.

Models	AIC	ΔAIC
Amount of cigarette butts x sex x breeding stage	**152.4047**	**0**
Sex x breeding stage	160.6505	8.2458
Amount of cigarette butts x sex + breeding stage	172.9074	20.5027
Amount of cigarette butts + sex + breeding stage	180.0322	27.6275
Amount of cigarette butts x sex + breeding stage	181.4212	29.0165

Table S11. Description of the best model to explain the amount of genotoxic damage in erythrocytes of *P. domesticus*. This analysis was made from only one individual per nest per sex (N=31; nest building n= 4 males, 4 females; incubation n= 4 males, 4 females; early breeding n= 3 males, 4 females; late breeding n= 3 males, 5 females).

Variable	Coefficient	SE	Z	*P*
Intercept	0.764	0.415	1.839	0.066
Amount of cigarette butts	7.937	2.541	3.124	0.002
Sex: females	1.306	0.480	2.720	0.007
Incubation	-0.767	0.753	-1.019	0.308
Early breeding	0.167	0.624	0.267	0.790
Late breeding	1.375	0.522	2.634	0.008
Amount of cigarette butts x sex: females	-4.509	5.090	-0.886	0.376
Amount of cigarette butts x breeding stage: incubation	0.136	4.074	0.033	0.973
Amount of cigarette butts x breeding stage: early breeding	-9.450	5.899	-1.602	0.109
Amount of cigarette butts x breeding stage: late breeding	-12.418	7.973	-1.557	0.119
Sex: females x breeding stage: incubation	0.732	0.822	0.890	0.373
Sex: females x breeding stage: early breeding	-0.388	0.729	-0.532	0.595
Sex: females x breeding stage: late breeding	-3.092	0.806	-3.838	<0.001
Amount of cigarette butts x sex: females x breeding stage: incubation	0.350	6.292	0.056	0.956
Amount of cigarette butts x sex: females x breeding stage: early breeding	3.523	9.079	0.388	0.698
Amount of cigarette butts x sex: females x breeding stage: late breeding	13.038	9.710	1.343	0.179