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Impact of road distance and experimental challenge of the maternal immune system on the offspring immunocompetence

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The increasing number of roads is an important threat to living organisms on a global scale, although research describing the influence of roads on the immune response of birds is lacking. The main purpose of this study was to examine the effect of distance from the road on the chicks' immune function. The research was conducted in the Krzyszkowice Forest near Kraków (S Poland) in spring 2012. Two common bird species: Great Tit and Eurasian Blue Tit bred in new nest boxes installed on trees with various distances from the road. In half of the nests, adult females were challenged with the antigen sheep red blood cells (SRBC) to check whether this experiment and the neighbourhood of road – both potentially lowering the immune response of nestlings – together cause an interactive effect. Nestling immune response was measured by the phytohaemagglutinin (PHA) skin-testing technique. Nestlings in nest boxes closer to the road showed a weaker reaction to PHA than those farther away. This effect was, however, noticed only in Great Tits' nests where the breeding female was not immune challenged. In both species, chicks raised by SRBC-challenged females had a low immune response irrespective of road distance. This research is the first one showing the relationship between indirect maternal effect, distance from the road and nestling immune response differing in two species.

1. Introduction

In recent decades, road building activity has dramatically increased on a global scale (Kuitunen *et al.* 2003) and European lands are especially heavily fragmented by roads (Psaralexi *et al.* 2017). The effects of roads on wildlife are mostly negative: they worsen the quality of habitat (Jaeger *et al.* 2005, Pescador & Peris 2007, Benitez-Lópes *et al.* 2010, Husby 2016), cause mortality (Erritzoe *et al.* 2003, Eberhardt 2009, Benitez-Lópes *et al.* 2010, Jack *et al.* 2015, Husby 2016), and decrease fledgling success (Kuitunen *et al.* 2003, Halfwerk *et al.* 2011, Holm & Laursen 2011). Roads are associated with the negative influence of four major environmental problems: noise, air pollution, light pollution and global warming (Dutta 2017). Only few studies show that roads have positive effects (Coffin 2007, Delgado *et al.* 2007, Polak *et al.* 2013, Morelli *et al.* 2014). Research concerning the effects of widely distributed roads on the immune function of birds is still lacking. This is a serious oversight, as the immune system is particularly expensive for organisms and causes trade-offs with other investments (Kerr *et al.* 2010, McNamara *et al.* 2013). The immune

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system is shaped mostly by the environment and ecological relationships (Tieleman *et al.* 2018), so roads can strongly affect this system.

Current knowledge about the impact of roads on the immune system can only be based on the known effects of factors related to roads, sometimes contrasting. Noise exposure alters physiological responses, decreasing population health (Shannon *et al.* 2016). More traffic noise may stimulate the immune system causing higher levels of haptoglobin plasma of chicks (Raap *et al.* 2017) but also suppress the immune function (Crino *et al.* 2013). Similarly, pollution can be immunosuppressive (Sanderfoot & Holloway 2017, Chatelain *et al.* 2018), but may also enhance the immune activity of birds (Audet *et al.* 2016, Watson *et al.* 2017).

The direct influence of roads on the immune system has not been studied so far. It is known, however, that early developmental stages are crucial to study the influence of the environment as they have long-term consequences on populations (Lindström 1999, Metcalfe & Monaghan 2001, Monaghan 2008, Biard *et al.* 2017). This research was conducted on the chicks' immune function to know the most urgent aspect of the unexplored impact of roads.

As mentioned, the immune system competes with other costly life-history traits, e.g. reproductive investment and maternal effects. Surprisingly, some studies show that noise exposure of adult females does not alter maternal effects (Injaian *et al.* 2019) and there is no maternal contribution to nestling immune transcript levels (Capilla-Lasheras *et al.* 2017). It has not been investigated if maternal effects impact the immune function of offspring developing near environmental disturbance after a challenge of the female immune response. Male Pied Flycatchers *Ficedula hypoleuca* show stronger humoral immune responses to a novel antigen (tetanus toxoid) in the vicinity of a copper smelter than in the unpolluted area (Eeva *et al.* 2005), which suggests that the immunological stimulation is greater under pollution, and likely also near roads. Immune-activation may also increase parental investment in offspring (Hanssen 2006).

On the other hand, the immune challenge of mothers may weaken the immune response of their nestlings (Grzędzicka & Kubacka 2018). We therefore need to integrate field research on the functional outcomes of immune challenge in relation to resource deterioration (Strandin *et al.* 2018). Proximity to roads can be a good proxy of resource deterioration and environmental degradation.

In this article, I examine the impact of a busy road on the immune response of chicks in Great Tit *Parus major* and Eurasian Blue Tit *Cyanistes caeruleus* breeding in the nest boxes, and the additional effect of experimental injection of adult females with a novel antigen sheep red blood cells (SRBC). I test the following hypotheses: (1) the offspring immunocompetence is lower in nest boxes installed closer to the roadway than in those hung farther away; (2) both road vicinity and maternal SRBC-response negatively affect the offspring immune response (Grzędzicka & Kubacka 2018) and cause an interactive effect. I expect that in the nests with female immune challenge the negative effect of road on chicks' immune response will not be detected e.g. due to increased maternal investment (Hanssen 2006); (3) the impact of road and maternal challenge on offspring immune function is species-specific (Grzędzicka & Kubacka 2018).

2. Material and methods

2.1. Study area

The study concerns Great Tits and Eurasian Blue Tits occupying nest boxes. At the turn of February and March 2012, a new research area was created with 170 nest boxes installed in the Krzyszkowice Forest near Kraków (S Poland). The area of this complex is 54.17 ha. The Oak-Hornbeam *Tilio-carpinetum* plant community dominates in the forest, some patches include also numerous Silver Birches *Betula pendula* and Scots Pines *Pinus sylvestris*. The forest habitat is not affected by distance from the road. No nest boxes were hung in the coniferous patch contrasting with the rest of the complex (Fig. 1). The distance between nest boxes was approximately 50 m; they were hung on trees about 2 m above the ground. After the first broods of birds in the year 2012, 110 of 170 nest boxes (65%) contained nests, while 60 boxes (35%) remained vacant. Nestlings were in 45 nest

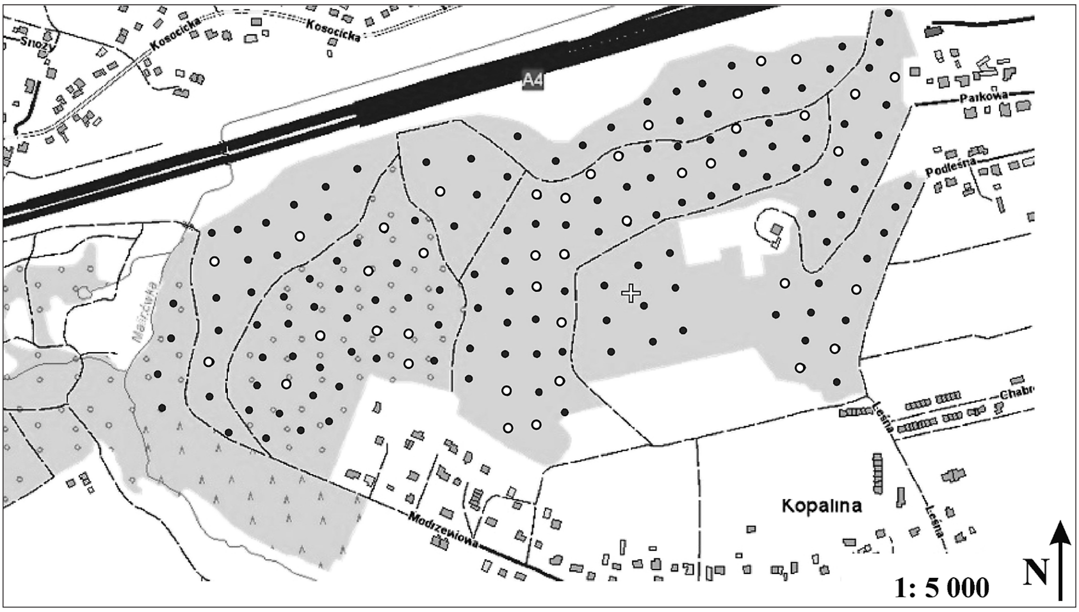

Fig. 1. Map of the research area in Krzyszkowice Forest; 170 dots are the locations of trees with nest boxes in the year 2012, where the locations of nest boxes randomly selected for bird injections were marked in white.

boxes occupied by Great Tits and in 35 nests of Eurasian Blue Tits. I conducted research involving injections on $N = 24$ (53% of 45) nests of Great Tits and $N = 16$ (46% of 35) nests of Eurasian Blue Tits.

One of the longer edges of the Krzyszkowice Forest is adjacent to the longest Polish roadway number A4, which in part near the research area was completed until the year 2009. There is a 15-metres green belt between woodland and roadway, composed of a neglected grassland and bushes. On this roadway, traffic activity is about 38.7 thousands of cars per 24 hours (data from measurements made in the year 2015 by the General Directorate of National Roads and Highways in Poland). This is the only busy road adjacent to the Krzyszkowice Forest, other roads in woodland marked on the map (Fig. 1) are forest paths not for cars.

The distribution of nest boxes, from which the breeding pairs were selected for the experiment ($N = 40$), was random (Fig. 1). I marked the locations of nest boxes on the map in the *Geoportal* program (*geoportal.gov.pl*) and measured shortest linear distances from each nest box location to the roadway in meters also in this program.

2.2. Field protocol

From the beginning of April, I checked nest boxes once a week, while in May 2–3 times per week. Since clutches were completed, boxes were checked every day after 13 days from the date of laying the first egg, to verify the predicted hatching date. Hatching date (May 1 = day 1) was the starting date for procedures in each nest. The study concerns only the first broods. The median hatching date was 9th May, the range of hatching dates was 1–17th May.

Three days after hatching, I captured breeding females using ornithological mist nets. The choice of nests for the experiment was random and both experimental groups were in older, younger, deciduous, and mixed patches of forest and across different hatching dates. In nests intended for experimental treatment ($N = 20$ nests), I immunised females with 0.1 ml of a 2% dilution of sheep red blood cells SRBC in a phosphate buffer saline PBS (procedure according to Deerenberg *et al.* 1997, Ros *et al.* 1997). SRBC initiates the innate and adaptive immune responses (Deerenberg *et al.* 1997, Cichoń *et al.* 2001, Ots *et al.* 2001, Hawley *et al.* 2005). The dose (2 mg of SRBC in the injected 0.1 ml volume of liquid) was constant for two species of tits, although Great Tit is about 30% heavier than the Eurasian Blue Tit (for more details about this choice in other population see: Grzędzicka & Kubacka 2018). In the control group (N = 20 nests), the injected substance was 0.1 ml of PBS. Both types of injections were performed intraperitoneally using an insulin needle.

The peak of the female immune response to SRBC is six days after injection (Snoeijs *et al.* 2007). Eleven days after hatching (and two days after this peak), I measured, weighed and injected nestlings with phytohaemagglutinin (PHA, Sigma L8754). This antigen affects T-cell-mediated immunity and is an indicator of cellular response (Brinkhof *et al.* 1999, Moreno *et al.* 1999, Hawley *et al.* 2005, Dubiec *et al.* 2006, Pickett *et al.* 2013, Podmokła *et al.* 2014).

Young birds were injected with 0.2 mg of PHA dissolved in 0.04 ml of phosphate buffer saline (PBS) in the right-wing web (Brinkhof *et al.* 1999, Smiths *et al.* 1999). I chose six nestlings per nest: two big, two medium and two small, based on their mass to correct for its effect on the nestlings' immune response. Differences in mass between chicks in each nest were small, as hatching in the Krzyszkowice Forest was fast and almost synchronous; usually lasted 1–2 days. Nestlings were ringed before the PHA-injection for individual identification. I took three repeated measurements of the wing web thickness for each bird before immunisation and 24 hours later with a pressure-sensitive specimeter (Mitutoyo 7313, measuring accuracy: ±0.01 mm).

The repeatability of measurements before the injection was: R = 0.83 in Great Tits and R = 0.94 in Eurasian Blue Tits. The repeatability of measurements after the immunisation was: R = 0.70 in Great Tits and R = 0.82 in Eurasian Blue Tits (R calculated according to the procedure by Lessells & Boag 1987). The difference between the mean pre- and post-injection wing thickness is defined as "wing web swelling". The wing web swelling data were collected from N = 24 Great Tit nests (12 with SRBC-injection and 12 control) and N = 16 Eurasian Blue Tit nests (8 females were challenged with SRBC, other 8 were injected with PBS).

2.3. Statistics

Kruskal-Wallis test was chosen to compare distances from the road between the trees with all nest boxes successfully settled by Great Tits and Eurasian Blue Tits in the study area (N = 80). The wing web swelling data were checked for normality using the Shapiro-Wilk test. Distribution of the PHA-response of nestlings of two species differed from a normal distribution (W = 0.947; P < 0.0001), so it was transformed into a normal distribution using the Continuous Fit function in the statistical programme *JMP 8* (Goos & Meintrup 2015).

I then used linear mixed-effects model LMM (Harrison *et al.* 2018), with wing web swelling of individual nestlings (N = 240, including 144 Great Tit nestlings from 24 nests and 96 Eurasian Blue Tit nestlings from 16 nests) as the dependent variable. The estimation method was restricted maximum likelihood (REML). Nominal independent factors were: "species" and "experimental treatment" (1 – female injected with PBS, 2 – female challenged with SRBC), continuous independent factors were: road distance, number of all nestlings per nest and their mass, while nest identity ("nest ID") was a random effect. Nestling mass and nestling number used in the model were measured just before PHA immunisation (eleven days after hatching). Also, four interactions were included as factors: "experiment by roadway distance", "experiment by species by roadway distance", "experiment by species", "species by roadway distance". Interactions were removed if non-significant and model was refitted.

The model fit was expressed by *R square* (*R2*), which measures the proportion of explained variance in the model. The remaining variation is attributed to random error (Harrison *et al.* 2018). The adjusted *R2* is used to select the best model and also to calculate a measure of fit (Johnson & Omland 2004). The fit of the model with four interactions was *R2adj* = 0.752, in the case of a model with three double interactions was *R2adj* = 0.753, while in the third model with two interactions *R2adj* = 0.754.

In the third final model, the power of the performed test and the least significant number (LSN), which are given for each factor, were estimated using effect sizes and standard errors from

Table 1. Results of the LMM model testing for the effects of roadway distance, hatching date, female experimental treatment (1 – PBS, 2 – SRBC), nestlings' mass and their number on the offspring PHA-response in the Great Tit (*N* = 144 nestlings from 24 nests) and the Eurasian Blue Tit (*N* = 96 nestlings from 16 nests). The fit of the full model: R^2 = 0.762. LSN is the number of observations (here: number of nestlings) needed to achieve a significant result assuming that the standard errors and effect size of the current sample remain the same.

Source of variation	Estimate	Standard error	*DF, DEN DF*	*F*	*P*	Power	LSN
Road distance	0.001	0.0002	1, 31.38	25.902	< 0.0001	0.999	39
Hatching date	–0.020	0.006	1, 34.02	13.229	0.0003	0.952	74
Experimental treatment [1]	0.124	0.018	1, 31.97	48.718	< 0.0001	0.999	23
Number of nestlings	–0.001	0.008	1, 31.95	0.029	0.865	0.053	3,254
Nestlings' mass	0.023	0.013	1, 231.1	3.142	0.078	0.423	302
Species [BT]	0.083	0.035	1, 38.2	5.563	0.019	0.651	171
Exp. [1] × road distance	–0.0005	0.0002	1, 32.5	11.131	0.001	0.913	87
Species [BT] × road distance	0.001	0.0002	1, 31.5	7.633	0.006	0.786	126

the sample data (Thusius *et al.* 2001). All of the above tests were performed in *JMP 8*.

Because the "experiment by roadway distance" and "species by roadway distance" interactions significantly affected the chicks' immune response, four additional models were designed in the *R 3.6.1.* software (R Core Team 2019) separately for each species with division into experimental groups. To test the influence of road distance on the individual nestling immunocompetence in each of these groups, four linear mixed-effects models LMMs were designed using the *lmer* function in the "lmerTest" package (Kuznetsova *et al.* 2017). In each model, the PHA-response of nestlings transformed to normality in *JMP 8* was a dependent variable with Gaussian distribution and identity link function, "nest ID" was a random factor, while the only continuous factor was a distance to the road. Model parameters were estimated via restricted maximum likelihood (REML).

Scatter plots showing the relationships between the offspring immune responses and distance to the roadway were prepared in *JMP 8*. The wing web swelling averages per nest were used for clarity of dependence. Results were considered significant if $P < 0.05$.

3. Results

Great Tits and Eurasian Blue Tits chose locations with different distances from the road (Kruskal-Wallis test: $H = 4.65$; $P = 0.031$; $DF = 1$; $N = 80$ nests). Great Tits occupied areas at longer distances from road (median = 210 m; $N = 45$), while Eurasian Blue Tits nested closer to the road (median = 150 m; $N = 35$).

The main LMM model (Table 1) showed a significant impact of distance from the road on the nestling wing web swelling but based on significant interactions this effect was different in each species and experimental group. Test power in the

Table 2. Results of the four additional LMM models showing the influence of road distance on the offspring wing web swelling, separately in each bird species and experimental group (PBS – control injection of female, SRBC – immune challenge of female)

	Estimate	Standard error	*DF*	*t*	*P*
Eurasian Blue Tit, control group (PBS)	0.005	0.055	1	0.090	0.934
Eurasian Blue Tit, experimental group (SRBC)	0.141	0.083	1	1.710	0.146
Great Tit, control group (PBS)	0.036	0.012	1	2.959	0.004
Great Tit, experimental group (SRBC)	–0.026	0.014	1	–1.778	0.078

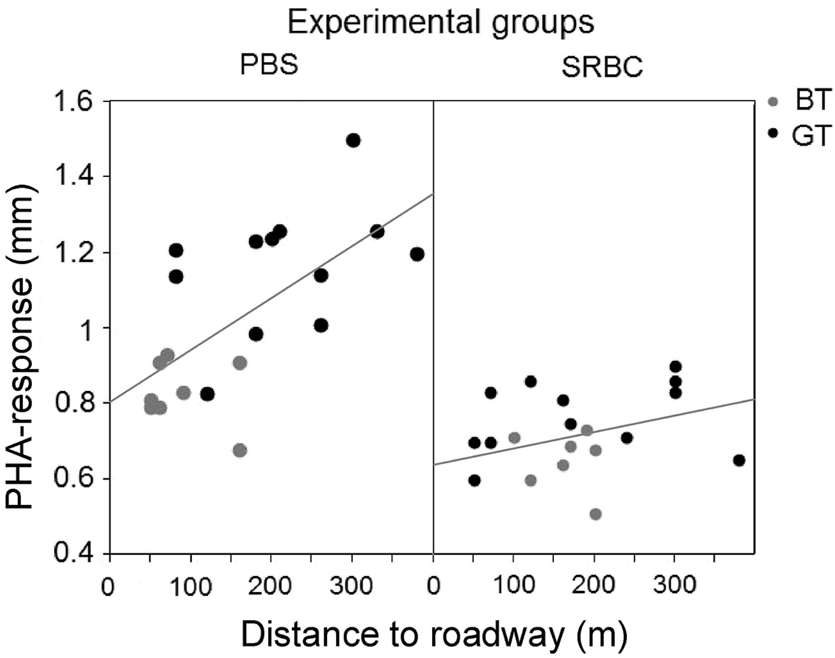

Fig. 2. Relationships between the offspring PHA-response (wing web swelling) and distance to the roadway in the control nests with a phosphate buffer saline (PBS) injection of the female and experimental nests with the sheep red blood cells (SRBC) challenge of the female of both Great Tit (GT) and Eurasian Blue Tit (BT). Points referring to Great Tits are black and to Eurasian Blue Tits grey.

case of interaction of the experiment and distance from the road was about 14% higher than in the case of interaction of the species with distance to the road (Table 1). According to four additional models (Table 2), in the control group without female immune challenge, the PHA-response of young Great Tits was positively correlated with distance from the road (the farther, the stronger), while in control Eurasian Blue Tit nests the road effect was insignificant (Table 2, Fig. 2). In nests where females were immunised with the SRBC antigen, the impact of road distance on the chicks' immune response was not significant in either bird species (Table 2, Fig. 2).

The main LMM (Table 1) showed also that the experimental challenge of females significantly affected the immune response of nestlings in both species. The offspring PHA-response was weaker in experimental nests than in control nests in Great Tit (SRBC-group: mean = 0.77 ± standard error = 0.03; PBS-group: 1.17 ± 0.05; Fig. 2) and Eurasian Blue Tit (SRBC-group: 0.65 ± 0.02; PBS-group: 0.83 ± 0.03; Fig. 2). The effect of "species" on nestling PHA-response was significant (Table 1), confirmed by the thinner wing web swelling in Eurasian Blue Tits, although this effect was about 35% weaker than in the case of "road distance" or "experimental treatment" impact. Hatching date was negatively correlated with the offspring PHA-response; chicks hatched earlier showed stronger reactions to the antigen. The nestlings' number and mass did not relate with their wing web swelling (Table 1).

4. Discussion

This study supports hypotheses concerning the influence of distance from the road and experimental female immunisation on the chicks' immune function. Chicks of control mothers had a higher immune response farther away from the road, which confirms previous studies indicating a negative impact of road infrastructure on birds (e.g.

Kuitunen *et al.* 2003, Jaeger *et al.* 2005, Pescador & Peris 2007, Benitez-Lópes *et al.* 2010). This work is the first one showing the negative impact of the road on the immune response, although it was significant only in Great Tits. In both species, chicks raised by SRBC-challenged mothers had a low immune response irrespective of road distance. It suggests the existence of an indirect maternal effect on the chicks' immunocompetence.

We can suspect that in nests where the females were immunised, chicks did not get the opportunity to develop a strong immune response to PHA even away from the road, where immunosuppressive noise (Crino *et al.* 2013) and pollution (Sanderfoot & Holloway 2017, Chatelain *et al.* 2018) were (probably) lower. After the female SRBC treatment, the offspring wing web swelling was low in all nests regardless of distance from the road. This result confirms previous studies that showed negative effects of the SRBC injection on birds (Deerenberg *et al.* 1997, Cichoń *et al.* 2001, Ots *et al.* 2001, Hawley *et al.* 2005, Grzędzicka 2018a, Grzędzicka & Kubacka 2018). In turn, the chicks raised by parents where the female was not immunised, at an appropriately long distance from the road were able to develop a stronger immune response. Therefore, this work suggests an effect of road distance on offspring immune responsiveness, although strongly affected by maternal immunisation. Road and female immune challenge are costly, and their interactive effect means that after immunisation there was no benefit of a long distance from the road to the chicks' immune function. Thus, it confirms that there is a trade-off between investment in life-history traits (reproduction) and immune system (Norris & Evans 2000, Carere *et al.* 2010).

The nestling PHA-response lower in nests near the road than in those located farther is an important result, as the reduced immune function is known to be negatively associated with survival rates of young birds (Hõrak *et al.* 1999, Christe *et al.* 2001, Menu *et al.* 2005). It is not surprising that bird nestlings suffer more from the effects of roads compared to adults as they are most sensitive to them and cannot move away (Crino *et al.* 2013) and effects of stressors in early life may be particularly detrimental (Casasole *et al.* 2017). Nevertheless, only a few studies published so far have shown the effect of environmental stressors on the young immune system. For example, chemicals exposure is associated with reduced immunocompetence in nestlings of Starlings *Sturnus vulgaris* (Markman *et al.* 2011). Thus, the results of this research concerning road impact on offspring immune function are an important and needed contribution to knowledge.

Although the positive correlation between immune response of chicks and distance from the road found in the control nests of Great Tit may be due to the direct influence of the road, the influence of other indirect factors cannot be excluded. Poor nestling immune condition close to the road can be explained by the content of harmful substances in caterpillars. Food items of tits also might have been affected by the road effects (Coffin 2007, Summers *et al.* 2011). Any contamination may both directly accumulate in the organisms and/or impoverish their food (Summers *et al.* 2011). On the other hand, this is contrary to the work showing that some chemicals are transported via water or wind and may affect wildlife at greater distances from roads (Forman *et al.* 2003). Caterpillar abundance in itself might be also different depending on road proximity and chicks near road could be in poor condition.

The conducted research confirmed the third hypothesis about species differences in the impact of roadway on the chicks' immune condition. It turned out that while in nests with experimental female immunisation, in both species the chicks' response to PHA was low and independent of the road, in the control group the road impact depended on species. In Great Tits, the immune response of young birds was stronger the farther from the road, while in Eurasian Blue Tits no similar effect was found. However, there was a significant difference between those two species in terms of distance from the road of occupied nest boxes.

The bigger Great Tit more often nested farther away from the road. The smaller Eurasian Blue Tit occupied nest boxes closer to the road and thus was potentially more exposed to the road impact. In the studied populations of birds, Great Tits also inhabited older forest patches than Eurasian Blue Tits (Grzędzicka 2018b), although habitat in Krzyszkowice Forest shows no variation depending on the distance to the road (personal observation). The difference between species in habitats was probably not associated with differences in the abundance of food, because diets of Great Tit and Eurasian Blue Tit nestlings did not differ in the proportions of caterpillars from various groups (Grzędzicka 2018b).

In populations that occupy nest boxes, however, the availability of suitable nesting sites poses an important selective force determining the behaviour of birds (von Haartman 1957, von Haartman 1971, Newton 1994). Eurasian Blue Tit is one of the smallest secondary cavity nesters in European forests; inferior in the nest competition with the larger Great Tit (Dhondt & Adriaensen 1999, Jacot *et al.* 2009). The inlets of nest boxes were optimal for the Great Tit, which could occupy nest boxes farther from the road by winning the nest site competition. In the case of Eurasian Blue Tit, occupying habitats closer to the road meant that all birds were exposed to it, which prevented the development of a stronger antigen response even in the control group.

We would expect that without the phenomenon of competition for nest boxes, Eurasian Blue Tits might occupy sites far enough from the road-related disorders so that chicks have the opportunity to develop a stronger response to PHA, which could result in a similar relationship as in the control nests of Great Tits. On the other hand, an artificially created research area consisting of nest boxes with inlets intended for the Great Tit reflects the conditions that occur in the natural environment. The Great Tit is usually the dominant species of tit in forest habitats, more numerous than the Eurasian Blue Tit (personal observation). Competition between them exists also outside areas with nest boxes (Dhondt & Adriaensen 1999, Jacot *et al.* 2009).

The differences found between species are not only the result of nest box area but may exist in nature. The necessity of nesting closer to roads may become the rule among "losers" similar to Eurasian Blue Tit. This means that the proximity of roads can play an additional selection role in populations losing the competition for a nest site or other resources. This result confirms the role of roads as a factor shaping adaptations and other evolutionary changes (Herrera-Montes & Aide 2010). The difference between species found in this study in terms of the impact of the road on the immune response of nestlings is – next to the interactive impact of the immune challenge of female and the distance from the road on the chicks' PHA-response – another interesting and novel result showing how a wide spectrum of effects roads can provide.

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Tien läheisyyden ja emon immuunivasteen vaikutus poikasten immuunipuolustukseen tiaisilla

Tieverkoston lisääntyvä määrä on merkittävä globaali ympäristöuhka. Tien läheisyyden vaikutuksia lintujen immuunipuolustukseen ei ole juurikaan selvitetty. Tutkimuksessa selvitettiin, miten tien läheisyys vaikuttaa poikasten immuunipuolustukseen tali- ja sinitiaisella. Tutkimus suoritettiin Länsi-Puolassa (Krzyszkowice Forest Kraków) sijaitsevassa populaatiossa, jossa pöntöt oli sijoitettu vaihteleville etäisyyksille tiestä.

Puolessa poikueista emoja immunisoitiin antigeenilla (SRBC), ja selvitettiin immunisaation ja tien läheisyyden yhdysvaikutusta. Tien läheisyyden oletettiin alentavan poikasten immuunipuolustusta. Poikasten immuunivaste mitattiin standardimenetelmin (phytohaemagglutinin, PHA-menetelmä). Tietä lähellä olevien pesien poikasilla oli heikompi PHA-immuunivaste kuin kaukana tiestä. Tämä efekti oli kuitenkin nähtävissä vain talitiaisella ja ei-immunisoitujen emojen poikasilla.

Molemmilla lajeilla immunisoitujen naaraiden poikasilla oli alempi immuunivaste, riippumatta pesän etäisyydestä tiehen. Tutkimus on ensimmäinen, jossa havaittiin yhteys epäsuoran äitivaikutuksen, tien läheisyyden ja poikasten immuunivasteen välillä eri lajeilla.

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