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VERSITA

Sources of variation in haematocrit in the Collared Flycatcher *(Ficedula albicollis)*

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Abstract The haematocrit rate of the blood shows the individual physiological state. As the haematocrit grows, the higher erythrocyte number results in more efficient oxygen uptake capacity which can lead to better performance and probably a better survival rate of an individual. Hence we assume that the high value of haematocrit reflects good health state. Altogether 308 blood samples were collected from a wild population of Collared Flycatchers *(Ficedula albicollis)* in two breeding stages during a period of 2008–2010. We tried to elucidate the relationship between condition and haematocrit level of an individual and studied the haematocrit changes of an individual between years. The haematocrit values differed between years. Females had higher haematocrit values than males in 2010 but not in 2009. At courtship the haematocrit level of males was higher, than during nestling care. The different environmental effects and energy demands of the individuals may be the driving force behind the observed changes in haematocrit level. Analysing the changes between two years, there was a positive correlation between changes in condition index and haematocrit of individuals. The haematocrit values of an individual were repeatable between years. This finding suggests that haematocrit can be informative about the individual's general health state.

Keywords: bird, blood, repeatability, health state, energy demand

Összefoglalás A vér hematokritértéke az egyed fiziológiai állapotáról nyújt információt. Feltehetőleg a magas hematokritszint jó egészségi állapotot tükröz, mivel a vörösvérsejtek megemelkedett szintje nagyobb oxigénfelvételi kapacitást és hatékonyabb oxigénszállítást tesz lehetővé a szövetekhez, ami az egyed jobb teljesítőképességét eredményezi. A Pilis hegységben 2008 és 2010 között odútelepeken költő örvös légykapókon *(Ficedula albicollis)* vizsgáltuk a hematokritérték évek és ivarok közötti eltérését, majd hímek esetében az udvarlás és a fiókanevelés stádiumában mért mintázatát. Vizsgáltuk az egyedek hematokritértékének és kondíciójának kapcsolatát. Továbbá számoltuk az egyedek hematokritértékének évek közötti repetabilitását. A hematokritértékek évek között több esetben eltértek. A hímek udvarlás alatti hematokritszintje magasabbnak bizonyult, mint fiókanevelés alatt. Az egyedek hematokritértékének repetabilitása magas volt, évek közötti eltérése pedig pozitívan korrelált kondíciójuk változásával. Feltehetőleg az évek közötti varianciát az eltérő környezeti feltételek okozhatták, míg az udvarlás alatt mért magas hematokritszint a megelőző vonulás nagy energiaigényének következménye lehet. Bár a hematokritérték változása volt megfigyelhető az évek és a szaporodási stádiumok között, az egyeden belül évek között mégis repetabilitást mutatott. Az egyedi hematokritértékek évek közötti repetabilitása lehetővé teszi, hogy a jelleg az egyed aktuális állapota mellett általános egészségi állapotáról is informáljon.

Kulcsszavak: madár, vér, repetabilitás, egészségi állapot, energiaigény

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Introduction

Haematocrit is a generally measured physiological object in human clinical processes, and it is emerging as a measure for the health state of individuals in natural populations (e.g. Hõrak *et al.* 1998, Ots *et al.* 1998, Hargitai *et al.* 2006, Kilgas *et al.* 2006).

Haematocrit shows the rate of packed cell volume to the volume of whole blood. Through the erythrocyte number haematocrit reflects oxygen uptake capacity and transport efficiency (Ots *et al.* 1998), which in turn could affect the survival of an individual. Low haematocrit level can indicate anaemia and associate with low available oxygen level (Phillips *et al.* 1985), therefore suggesting that birds may use anaerobically metabolized energy which may impair flight performance.

Birds can increase their haematocrit level as an adaptive response when their energy demand grows and more oxygen uptake is needed. This was shown in a brood size manipulation study of Great Tits *(Parus major)* (Hõrak *et al.* 1998) and a tail elongation study of Barn Swallows *(Hirundo rustica)* (Saino *et al.* 1997a). Both interventions resulted in increased haematocrit level.

Many studies assume that haematocrit is a good and reliable fitness related trait because it can reflect the condition and disease status of an individual (e.g. Svensson & Merilä 1996, Saino *et al.* 1997a, Sánchez-Guzmán *et al*. 2004). However, haematocrit can change very quickly as the effect of changing energy demand or different environmental factors (Fair *et al.* 2007), such as food availability (Merino & Potti 1998, Hoi-Leitner *et al.* 2001, Santangeli *et al*. 2012) or parasite infection (Heylen & Matthysen 2008, Palinauscas *et al*. 2008). Hence, there is a dispute about its potential to reliably reflect individual quality.

Our goal was to examine the patterns of haematocrit in a wild population of Collared Flycatchers: (1) to compare the within- and between-year variation in haematocrit among sexes, (2) to test the relationship between the condition index and the haematocrit of an individual and (3) to analyse the within-individual repeatability of haematocrit between years.

Materials and Methods

Study area and field methods

Our study was carried out in the Pilis Mountains (47°43’ N, 19°01’ E), located 30 km North of Budapest in Hungary. We studied a population of Collared Flycatchers breeding in artificial nest-boxes in an oak-dominated woodland from 2008 to 2010. The Collared Flycatcher is a long-distance migratory, insectivorous passerine.

We caught the individuals in their nest-boxes. Males were captured twice: firstly, during courtship, after arrival from migration, when they occupy nest-boxes (21 and 87 males in 2008 and 2010, respectively) and secondly, during nestling care, when nestlings were 6 days old (41 and 71 males in 2009 and 2010, respectively). Females were captured during nestling care (41 and 47 females in 2009 and 2010, respectively).

A blood sample (50–70 µl) was taken from the brachial vein into heparinized capillaries within 20 minutes of capture and centrifuged at 10.000 r.p.m. for 10 minutes on the same day. The haematocrit value was calculated as the ratio of the length of the capillary occupied by red blood cells and the length of the capillary occupied by total blood.

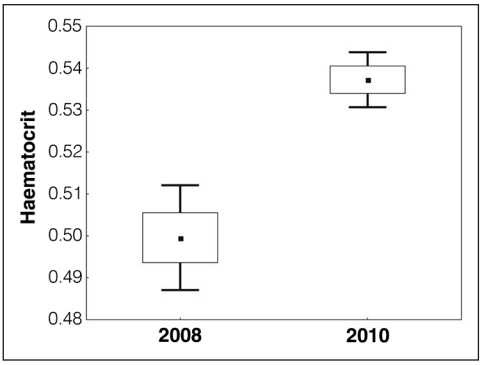

Figure 1. The haematocrit values of male Collared Flycatchers during the courtship in 2008 and 2010
n_{2008}=21; n_{2010}=87
Note: on the boxplot: the points are the means of the values, the squares show the standard errors, and 95% confidence intervals are presented as whiskers.

1. ábra Örvös légykapó hímek udvarlási periódusban mért hematokritértékei 2008-ban és 2010-ben
n_{2008}=21; n_{2010}=87
Megjegyzés: A boxplot ábrán a pont az értékek átlagát mutatja, a négyzet a standard hibát ábrázolja, a vonallal jelzett tartomány a 95%-os konfidencia intervallumot jelöli.

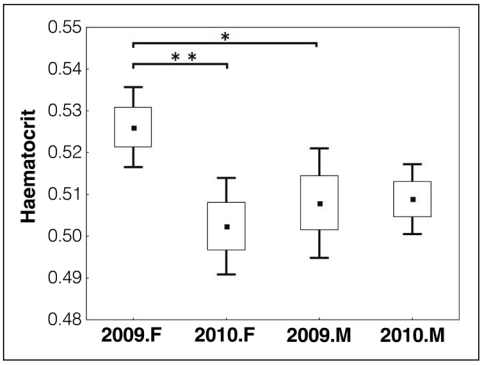

Figure 2. The difference in haematocrit levels between years and sexes (F=female; M=male) during nestling care
$n_{2009, female}$=41; $n_{2010, female}$=46; $n_{2009, male}$=41; $n_{2010, male}$=70
*Note: for the explanation of the boxplot see Figure 1 * p=0.027; ** p=0.0025*

2. ábra A hematokritértékekben megmutatkozó évek és ivarok közötti különbség (F=-tojó; M= hím) nevelési fázisban
$n_{2009, tojó}$=41; $n_{2010, tojó}$=46; $n_{2009, hím}$=41; $n_{2010, hím}$=70
*Megjegyzés: a boxplot magyarázatát lásd 1. ábra; * p=0,027; ** p=0,0025*

Birds were weighed with Pesola spring balance (to the nearest 0.1 g) and the length of the tarsus was measured with a sliding calliper (to the nearest 0.1 mm). Body condition was calculated as the residual of a linear regression of body mass on tarsus length.

Statistical analyses

We performed all statistical analysis in the R software (version 2.12.2[1]). Unless stated otherwise, we used linear regression models and Pearson correlation to test the interrelation of variables. We calculated the change in haematocrit and condition by subtracting their values in 2010 from the values in 2009. We estimated the repeatability of haematocrit between years using the method developed by Lessels and Boag (1987).

Results

During courtship, the haematocrit level of males was higher in 2010 than in 2008 (F=27.02; df=106; p<0.0001; *Figure 1*).

During nestling care, we studied the variation in haematocrit level between 2009 and 2010 and between the sexes *(Figure 2)*. Comparing the years separately by sexes, haematocrit level of females showed a higher level in 2009 than in 2010 (F=9.75; df=84; p=0.0025). Years did not have any significant effects on the haematocrit level of males (F=0.016; df=109; p=0.899). Comparing the sexes in both years, we found that females had higher haematocrit values than males in 2009 (F=5.057; df=79; p=0.027), but there was no difference between sexes in 2010 (F=0.866; df=114; p=0.35; *Figure 2*).

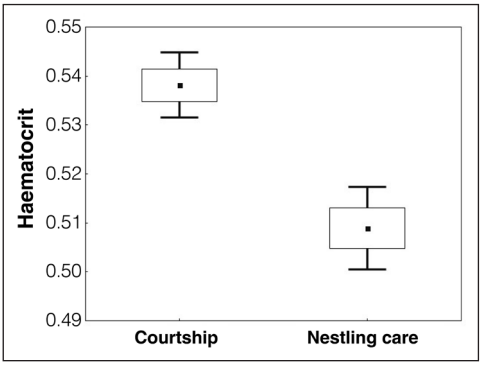

Figure 3. The haematocrit levels of male Collared Flycatchers during courtship and nestling care, in 2010
$n_{courtship}$=70; $n_{nestling\ feeding}$=87
Note: for the explanation of the boxplot see Figure 1

3. ábra Örvös légykapó hímek hematokritértékei udvarlási és fiókanevelési stádiumban, 2010-ben
$n_{udvarlás}$=70; $n_{fiókanevelés}$=87
Megjegyzés: a boxplot magyarázatát lásd 1. ábra

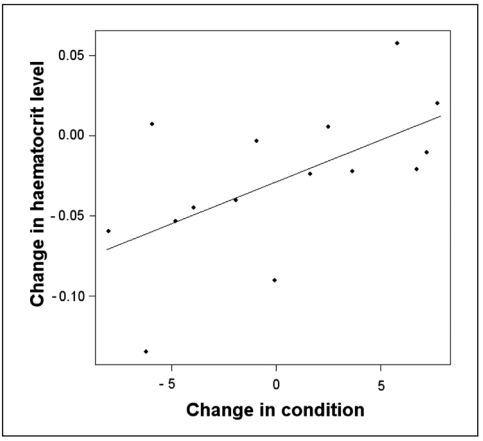

Figure 4. The relationship between the change in condition and haematocrit level of individuals, between 2009 and 2010. Traits were measured at nestling feeding period
n_{female}=6; n_{male}=9

4. ábra 2009-ben és 2010-ben nevelési stádiumban visszafogott egyedek hematokrit- és kondícióváltozásának kapcsolata
$n_{tojó}$=6; $n_{hím}$=9

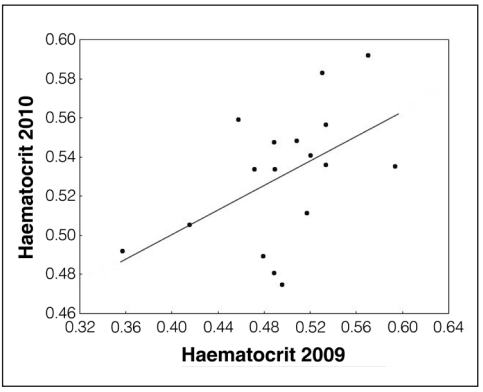

Figure 5. The repeatability of individual haematocrit levels between years. Birds were captured in 2009 and recaptured in 2010, during nestling care
n_{female}=6; n_{male}=11

5. ábra Az egyedi hematokritértékek évek közötti repetabilitása. Fiókanevelési stádiumban 2009-ben mért, majd 2010-ben visszafogott madarak alapján
$n_{tojó}$=6; $n_{hím}$=11

In 2010, blood samples were taken from males in two breeding stages which revealed a higher haematocrit level during courtship than during nestling care ($F=33.48$; $df=123$; $p<0.0001$; *Figure 3*).

As differences between years, sexes and breeding stages were detected in haematocrit, the correlation between the condition and the haematocrit level of individuals was analysed separately for years and sexes during nestling care. No correlation was found between the two individual traits (all $p>0.5$; *Table 1*).

However, the change in condition was positively correlated with the change in individual haematocrit level between 2009 and 2010 during nestling care ($F=7.086$; $df=13$; $p=0.02$; *Figure 4*). Sexes were examined together.

The haematocrit values of birds captured in 2009 and also in 2010 during nestling care showed high repeatability between years (repeatability: 0.676; $p=0.038$; *Figure 5*).

	2009				2010			
	Estimate	SE	t	p	Estimate	SE	t	p
Male condition	<0.001	0.002	0.1	0.921	<0.001	0.001	-0.58	0.564
Female condition	<0.001	0.001	0.08	0.936	<0.001	0.001	-0.16	0.875

Table 1. The relationship between condition and haematocrit level of individuals. The analyses were made separately for years and sexes during nestling care

1. táblázat A madarak kondíciójának és hematokritértékének kapcsolata fiókanevelési stádiumban mérve, ivarok és évek szerint

Discussion

To shed light on the sources of variation in haematocrit in the investigated wild population of Collared Flycatcher, firstly, we compared the haematocrit levels between years. The differences we have found between years support the sensitivity of haematocrit to environmental factors. Comparing birds captured during courtship in 2008 and 2010, the haematocrit level significantly differed. The difference between 2009 and 2010 was also significant when females during nestling care were compared, but not in the case of males.

The genetic and environmental components of the variation in haematocrit were previously examined in Barn Swallows (Cuervo *et al.* 2007) and Pied Flycatchers *(Ficedula hypoleuca)* (Potti *et al.* 1999). According to the cross-fostering experiments the measured variation in haematocrit level of the fledglings in these species is explained more by the nest where they were reared than the nest to which they genetically belonged.

The environmental influence on haematocrit level was demonstrated in experiments where increased food availability resulted in increased haematocrit level (Merino & Potti 1998, Hoi-Leitner *et al.* 2001, Santangeli *et al*. 2012), while fasting birds decreased their haematocrit values (Boiesmenu *et al.* 1992, Merilä & Svensson 1995). Török *et al.* (2004) studied the varying food availability in our study site between years. One of the main food type of the Collared Flycatcher is the caterpillar, which showed a high biomass variance estimated by caterpillar frass collection (Török *et al.* 2004). Further experimental studies are required to assess the role of the environmental factors in the variance of haematocrit level.

Literature is contradictory about the fact whether the sexes differ in terms of haematocrit (e.g. Hõrak *et al.* 1998, Potti *et al.* 1999, Fair *et al.* 2007). We found ambiguous patterns in our flycatcher population, as well, with a difference between sexes in 2009 but not in 2010.

As mentioned before, the haematocrit level can change with growing energy demand. Prior to feeding the nestlings, the nest building, egg laying and incubating all belong to the female's investments, which could have an effect on the high haematocrit level of females in 2009. However, males occupy and guard nest-boxes, and after the hatching both parents feed the nestlings, which could cause similar haematocrit rates between sexes.

Beside the effect of the changing energy demand, erythropoesis is generally suppressed during egg laying, causing reproductive anemia by females (Sockman *et al.* 2006, Wagner *et al.* 2007, 2008, Willie *et al.* 2010). Haematocrit decreases to the first-egg stage and the recovery is relatively

long-lasting, extending through incubation and hatching period (Wagner *et al.* 2008). This recovery can be regulated by high plasma prolactin level which is typical during incubation (Sockman *et al*. 2006). In an experimental study, increased prolactin caused increased haematocrit in hypophysectomized pigeons (Höcker 1969). Anti-estrogen (tamoxifen) treatment reduced the decrease in haematocrit during egg production in female Zebra Finches, which indicates that the decrease in haematocrit may have been due to antagonistic pleiotropic effects of estrogens (Wagner *et al.* 2007). Females captured during nestling feeding should be in recovery phase from anemia, which would mean a relatively low haematocrit level, something we did not observe when compared to males. However the postnuptial moult usually commences earlier in males than females, and may partly overlap with nestling feeding. During moulting the haematocrit level is decreasing (Chilgren & deGraw 1977, Driver 1981, Merino & Barbosa 1996). The presumably increasing haematocrit level of females during recovery phase and the decreasing level of males during early postnuptial moulting could equate the haematocrit level of sexes during nestling feeding. We did not find any effect of the sexes on haematocrit in 2010, as Potti *et al.* (1999) also did not find a difference between sexes by studying a sister species, the Pied Flycatcher. These stage-specific factors may have environment-dependent effects on the haematocrit level of the sexes which may have led to the sex difference in haematocrit in one year but not in another.

In 2010 we captured males during courtship and also during nestling care. Comparing the haematocrit values between the two breeding stages, we found that during the courtship the haematocrit level was significantly higher. We captured males at courtship a few days after arrival from migration. Because haematocrit is proportional to the metabolic activity during periods of days before blood sampling (Carpenter 1975, Ots *et al.* 1998), the high energy demand of long distance migration could have caused the increased haematocrit level by males sampled at courtship. Landys-Ciannelli *et al.* (2002a) pointed out the haematocrit-increasing effect of long-term flying with Bar-tailed Godwits *(Limosa lapponica)*. In addition to the highly increased energy demand of long-term migration, hormonal changes could also affect the haematocrit level. Several studies have shown the growing plasma corticosterone level just before and during migration (Holberton 1999, Piersma *et al.* 2000, Landys-Ciannelli *et al.* 2002b), which could directly increase the haematocrit level of birds. The testosterone level of males is also increasing until courtship and has the highest level during the mating period. During the breeding period this level must decrease, because high testosterone holds back the nestling feeding mechanisms (Wingfield *et al*. 1990). Experimental studies have shown that testosterone treatment increases erythropoesis and haematocrit level in some bird species (Domm & Taber 1946, Robinzon & Rogers 1979, Thapliyal *et al*. 1983). In another study, testosterone and haematocrit were simultaneously growing with sexual maturity of the male turkey (Cecil & Bakst 1991). In line with our findings, the decreasing haematocrit level during the breeding period was also experienced by Barn Swallows (Saino *et al.* 1997b). The observed differences between the two breeding stages may be due to the highly increased energy demand of long-term migration and the changing level of the hormones testosterone and corticosterone.

We assumed that haematocrit reflects the health state of an individual and could play a role in survival. Based on this, we expected a positive correlation between condition and haematocrit level of individuals. However, these traits were not correlated with each other.

Due to the life history trade-off theory, we would expect a negative relation between two costly fitness-related traits. However, because of individual optimisation and because probably both of the studied traits are related to fitness, we could also expect a positive correlation. Still, it happens that fitness related traits do not correlate with each other. Hegyi *et al.* (2002) showed that forehead patch size is unaffected by body condition in the same population of Collared Flycatcher that we studied. Even so, both of the traits can reflect individual fitness. In a Swedish population of Collared Flycatchers the forehead patch size was positively related to lifetime reproductive success (Gustafsson *et al*. 1995). Two uncorrelated fitness-related traits could reflect different aspects of the complex individual quality.

A significantly positive relationship appeared in the change of haematocrit and condition, which may partly be due to the possible quick changes in their value. Both of them are capable of reflecting the current physiological status of individuals. Sánchez-Guzmán *et al.* (2004) found the same relationship in Northern Bald Ibises *(Geronticus eremita)*, and Rattner *et al.* (1987) showed a relationship between the growing weight and the haematocrit level of American Black Duck fledglings *(Anas rubripes)*.

When we compared the haematocrit levels of birds captured in two different years but in the same breeding stage, the haematocrit of individuals showed high repeatability. Studying a Pied Flycatcher *(Ficedula hypoleuca)* population, Potti *et al.* (1999) also found that haematocrit was repeatable between years.

To summarize, our results indicate the sensitivity of haematocrit: (1) to environmental factors, indicated by differences between years, and (2) to growing energy demand and the hormonal background, suggested by the high haematocrit value after migration, at courtship. We observed that a relationship with condition only appeared when we compared the changes of the traits, possibly due to the combination of the short-term flexibility of the two traits and their similar sensitivity to large-scale environmental factors. Finally, possibly our most interesting result is that despite its variability, the individual's haematocrit was repeatable between years. This finding suggests that haematocrit may not only reflect the current status of an individual (condition change associated with haematocrit change), but it may also be capable of informing us about the individual's general health state.

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[1]http://cran.r-project.org/bin/windows/base/old/2.12.2/