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Title:

Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood

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Abstract

Haematological methods are widely utilized among avian ecologists as a means for individual health assessment. However, the technical simplicity of some of the tests may easily lead to oversimplification of the evaluation. Here we show in the Scarlet rosefinch (*Carpodacus erythrinus*) that haematological parameters other than the widely used heterophil/lymphocyte (H/L) ratio may be important to investigate. We give the full description of seven basic haematological traits (leukocyte differential count, immature erythrocyte count, haematocrit, mean cell volume, total red and white blood cell count and blood parasite occurrence). Most remarkably, the examination of 178 adults and 155 nestlings has revealed that this species has an extraordinarily high proportion of basophils in the peripheral blood (on average about 42% and 56% respectively). Although the high basophil count is a general trait even in healthy individuals of this species, the proportion of these cells is condition-dependent and is further increased by *Haemoproteus* infection. Our results also suggest that the immature erythrocyte count in the peripheral blood is a good predictor of the nestlings' growth rate. We conclude that the rosefinch haematology differs strikingly from other avian species with known values of basic haematological parameters. We therefore emphasize the importance of a general haematological examination, based on material obtained by an appropriate method (e.g. for smear preparation, we recommend using differential staining and avoiding prior methanol fixation).

Key words

Avian immunity, basophilic granulocyte, hematology, hematocrit, leucocyte differential count, packed cell volume, polychromatic erythrocyte.

Introduction

Health has a major effect on body condition and vigour, which consequently determines individual fitness. Adoption of appropriate methods that enable a reliable estimation of health is therefore of crucial importance to most ecological and evolutionary research. Although there are various ways to investigate health, the most widely used method for health assessment is the basic haematological survey (Ardia and Schat 2008). The main procedure for a haematological survey is to determine the cellular composition of blood. In avian peripheral blood, there are five morphologically distinguishable types of leukocytes present: lymphocytes, heterophils, basophils, eosinophils and monocytes (Lucas and Jamroz 1961). Normal values of cellular proportions may differ among species (Campbell and Ellis 2007; Davis 2009) and may also vary between free-living and captive-held birds (Ewenson et al. 2001). In most bird species with published values of haematological traits, however, only lymphocytes and heterophils are detected in sufficient numbers to enable reliable interindividual comparison (the combined number of lymphocytes and heterophils typically accounts for 85-95% of all leukocytes in a blood smear; Davis 2009). Several studies have documented that the ratio of lymphocytes and heterophils (H/L) may reliably indicate stress (El Lethey et al. 2003; Davis et al. 2008) as well as infection status for some diseases (Davis et al. 2004; Chakarov et al. 2008; Fokidis et al. 2008; Norte et al. 2009a). These findings have led avian ecologists to use the H/L ratio as general measurement of health status, which is quick, repeatable, and easy to obtain (Ots et al. 1998; Ardia and Schat 2008).

Despite the growing number of haematological studies in birds (e.g. see Moreno et al. 1998; Hauptmanová et al. 2002; Uhart et al. 2003; Sergent et al. 2004; Friedl and Edler 2005; Davis et al. 2004; Mercurio et al. 2008) data on blood cellular composition are still only available for a limited number of passerine species. In contrast to the known variability (e.g.

interspecific or linked to age and sex) and fluctuations (e.g. due to stress, season or health changes) in heterophil and lymphocyte counts, the variability among other cell types is often considered as only minor and rare (Maxwell 1981; Dufva and Allander 1995; Scope et al. 2002; Dubiec et al. 2005; Lobato et al. 2005; Davis 2005, Norte et al. 2009a; Norte et al. 2009b). Nevertheless, certain illnesses induce quantitative changes in the number of basophils, eosinophils and monocytes that are comparable to the changes observed in lymphocytes and heterophils (see e.g. Fudge 1989; Campbell and Ellis 2007).

Detailed studies of the composition of avian blood cells can offer more information than is usually considered. Aside from the common methods of estimating proportions of leukocytes and quantities of blood-borne cells (described either as absolute counts or as haematocrit; Ots et al. 1998), determining the blood cell dynamics may be equally valuable. For instance, immature erythrocyte count can reveal the presence of anaemic diseases (Campbell and Ellis 2007) and there is some toxicological evidence suggesting potential importance of this trait for wildlife studies (see Yamato et al. 1996; Belskii et al. 2005; Carleton 2008). However, to our knowledge there has been no effort in avian ecology to examine the health-predictive potential of peripheral blood immature erythrocyte count in free-living birds.

In this study we investigated the basic haematological traits in the Scarlet rosefinch (*Carpodacus erythrinus*). The Scarlet rosefinch is a small sexually dichromatic passerine belonging to the subfamily Carduelinae (Cramp et al. 1994). A remarkable feature of this species is the especially long distance and direction of its migration. The wintering grounds are located in the southern part of Asia (Cramp et al. 1994), lying in a distance of approximately 5 500 km from the breeding grounds in central Europe. Thus, the energetically costly migration may be expected to pose a powerful selection on health related traits in this

species (Albrecht et al. 2007). Here we present data on the leukocyte differential count, total counts of leukocytes and erythrocytes, haematocrit, blood parasites, and the proportion of immature blood cells. Most of the traits are reported in adults as well as in seven-day-old hatchlings. Chosen haematological traits were correlated with morphological condition-dependent traits and blood borne parasite infection status to assess their convenience for general health prediction in this species.

Materials and methods

Field procedures

The research was carried out on a Scarlet rosefinch population breeding in the Vltava river valley, Šumava National Park, southern Bohemia, Czech Republic (N 48°48'–48°50', E 13°55'–13°57', ~730 m above sea level; for more detailed description see Albrecht 2004). This population numbers at least 200 breeding pairs (Šťastný et al. 2006) and represents the western edge of the breeding grounds for this species (Risberg and Stjernberg 1997). In five successive seasons (2004-2008) we examined the basic haematological traits of 178 Scarlet rosefinch adults (110 males, 68 females) and 155 nestlings (not all traits were examined in every individual).

In each season samples were collected during the pre-breeding (second half of May) and breeding (second half of June) periods. Adult birds were captured into standard ornithological mist nets, either with the lure (stuffed male Scarlet rosefinch skin) or by chance in the surrounding of their putative breeding spot. After capture, each bird was placed into a fabric bag, weighed using a spring balance (MicroLine 20060, 60g, d=0.5g; Pesola AG, Baar, Switzerland) and a sample of blood (about 20-70µl) was collected by puncturing the brachial vein. Thereafter the tarsus length was measured by digital calliper (accuracy 0.01mm) as a

general estimate of individual's size (Senar and Pascual 1997). Individual weight was later divided by tarsus length and this weight standardized on size (hereafter referred as mass) was used for further analyses as a condition indicator. When all required information was collected the bird was tagged with a standard steel ring from the Czech ringing station (N MUSEUM PRAHA) and released. The manipulation time was about twenty minutes in total.

Every year during the breeding season the Scarlet rosefinch nests were systematically searched. At the day of hatching each nestling was individually marked with a permanent marker and the hatching date was recorded. On day 7 post hatch (i.e., determined individually for each nestling within the nest according to its hatching date), a blood sample (ca. 10-30μl) was collected from each nestling. Thereafter the nestling was tagged with a darkened steel ring, weighted and the tarsus length was measured. On the next day the nestling was measured again to obtain the measure of nestling growth (tarsus length on day 8 post hatch minus the length on day 7 post hatch). Sampling of nestlings was performed in a hidden place about 20m away from the nest to minimise the risk of attracting a predator. No nestling spent more than 30 minutes out of the nest.

Leukocyte differential count

In all adults as well as in all nestlings a drop of blood was used immediately after collection for the preparation of a blood smear. The air-dried smears were not fixed in methanol (as usual in mammalian haematology) before staining because methanol fixation decreases the stainability of basophil granules, which impairs correct identification of basophils (Robertson and Maxwell 1990, personal experience; see also Dubiec et al. 2005). After transportation to laboratory all smears were stained by Pappenheim's panoptical method: 3 minutes in concentrated May-Grünwald staining solution (Merck & Co., Inc. Whitehouse Station, NJ,

USA), 2 minutes in the same solution diluted 1:1 with distilled water and 15 minutes in Giemsa's solution (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:40 with distilled water, then washed with water and air dried. This differential staining method enables the recognition of all basic blood cell types under a light microscope. All smears were scanned with Olympus CX-31 microscope (Olympus Corporation, Tokyo, Japan) under magnification of 1000x to count the proportions of lymphocytes, heterophils, eosinophils, basophils, monocytes and immature leukocytes within a sample of 110-140 leukocytes in the smear. Being morphologically similar to leukocytes described from other avian species individual leukocyte types were identified according to Lucas and Jamroz (1961) and Campbell and Ellis (2007). The immature stages of all leukocyte types found in the peripheral blood were assigned to a single category due to the potentially high risk of incorrect identification of the cell type. The repeatability of measuring the leukocyte frequencies was calculated based on a sample of 27 adult individuals for which counts were taken two separate times. It was r=0.87 for lymphocytes, r=0.91 for heterophils, r=0.89 for basophils, r=0.71 for eosinophils, r=0.32 for monocytes and r=0.33 for immature cells. The low values for repeatability in monocytes and immature cells were caused by their low frequencies among blood leukocytes (on average less than 5% in both cell types).

Erythrocyte differential count

In 20 randomly chosen adults and 20 nestlings, we measured the differential count of immature erythrocytes. In each individual blood smear 5 randomly chosen monolayer fields were photographed (camera E-410; Olympus Corporation, Tokyo, Japan) at 100x objective magnification (ca. 1000-2000 cells). Copies of all images were transformed into black and white 1 bit format with resolution 80 dpi by Corel PHOTO-PAINT X3 software (Corel Corporation, Ottawa, Canada) so that only the uniformly black nuclei remained visible.

Where necessary, the neighbouring nuclei were manually separated and potential dirt was deleted. The total number of cells per image was automatically counted from these adjusted images by the particle analyser of ImageJ software (Rasband W.S. ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997-2008). The original photographs were then used to manually count the immature erythrocytes, thrombocytes and leukocytes within the images using ImageJ cell counter. Within erythrocytes we distinguished among normal mature erythrocytes, immature polychromatic erythrocytes and hypochromatic cells (for detailed description see Campbell and Ellis 2007). The number of reticulocytes has not been determined as this would require a special type of staining (Campbell and Ellis 2007). In samples from the adults, but not the nestlings, it is not clear whether the hypochromatic erythrocytes represent immature ontogenetic stages or rather aberrant cells (Campbell and Ellis 2007). Therefore only polychromatic cells were counted as immature erythrocytes in adults (r=0.89) while in nestlings a join category of polychromatic and hypochromatic cells was created (r=0.95).

As the onset of degenerative processes in thrombocytes may take place rapidly after the blood collection and variably among individuals (Lucas and Jamroz 1961), no thrombocyte counts were recorded.

Haematocrit

The haematocrit (packed cell volume) values were recorded in 56 adults. Blood samples were centrifuged in heparinised capillary tubes for 5 min. at 11000 rpm. Then the proportion of cells in the total volume of blood was measured and recorded as a percentage. The repeatability of the measurement was r=0.97, estimated based on a sample of 17 individuals in which two capillary tubes of blood were collected. The haematocrit measurement was not

carried out in nestlings as it requires the collection of an additional 40-50µl of blood, which exceeds the volume of blood that can be safely taken.

Total leukocyte and erythrocyte count

In 20 adult rosefinches captured in 2008, we examined the total red blood cells count (i.e. number of erythrocytes per volume unit of blood, hereafter TRBC), and the total white blood cells count (i.e. number of leukocytes per volume unit of blood, hereafter TWBC). A blood sample of 15µl was diluted in 2985µl of Natt and Herrick‘s solution (for composition description see Campbell and Ellis 2007) and left for several hours in a field refrigerator to stain the cells. Thereafter the numbers of cells were counted in the Bürker’s counting chamber (100 large squares for leukocytes and 20 rectangles for erythrocytes were scanned). Neither TRBC nor TWBC was investigated in nestlings to avoid any potentially harmful effect of larger blood volume losses on their ontogeny. Haematocrit and erythrocyte count values were used for calculation of the mean cell volume (MCV) as a quotient of these two multiplied by 10 (Campbell and Ellis 2007). Since 16 out of the 20 samples that we measured TRBC and TWBC came from males, we investigated the relationship of these parameters to condition only for this sex.

Total and differential counting of erythrocytes and leukocytes as well as all other haematological examinations were performed by one person only (MV) to minimise any potential variability among the measurements.

Parasite prevalence

The method for blood parasite load assessment has been previously described in detail elsewhere (see Votýpka et al. 2003) and so here we report it only briefly. The blood smears

were examined with a light microscope at 200x magnification for 5 minutes (approximately equivalent to the observation of 50 microscopic fields). Each smear was thereafter examined for another 10 minutes at 1000x magnification (equivalent to 100 microscopic fields; minimally 10 000 erythrocytes). When no parasites were detected after this time, the smear was considered as negative.

Statistics

For comparisons of sexes and age classes the Two-sample t-test was performed where possible (i.e. when the variable had a normal distribution or when normality was achieved by transformation). However, as cell frequencies often did not possess Gaussian distribution, nonparametrical Wilcoxon rank-sum test had to be adopted. Correlations were tested by Pearson's product-moment correlation. Generalized linear models were used for the haematological-condition analyses. In these models the distribution was approximated to Gaussian (e.g., in the analyses of basophils in adults and immature erythrocytes) or to binomial (e.g., in the analysis of H/L ratio in adults). We used generalized linear mixed effect approach to test the influence of nestling condition on its H/L ratio and basophil count. In this analysis, the identity of nests was treated as a random effect. Minimal adequate models, i.e. models with all terms significant (Crawley 2002) were obtained by backward eliminations of particular terms in candidate models using likelihood ratio approach starting with the most complex terms (Crawley 2002). The significance of a particular term adjusted for the effects of other terms was based on the change in deviance between the full and reduced models, distributed as χ^2 with degrees of freedom (df) equal to the difference in the degrees of freedom between the models with and without the term in question. F statistics rather than χ^2 statistics were used in cases of overdispersion. All presented significance values are based on the Type III Sum of squares. In all cases the reliability of the sample distribution

approximation to the chosen distribution was tested by One-sample Kolmogorov-Smirnov goodness-of-fit test. The significance level was set to p=0.05. Repeatability was assessed according to Lessells and Boag (1987). All statistical analyses were performed using S-PLUS 6.0 (Lucent Technologies, Inc, USA) and R 2.8.1. (http://www.r-project.org/) softwares.

Results

Basic description of the Scarlet rosefinch haematology

The leukocyte differential count in adult individuals is summarized in Table 1. In the adult rosefinches, haematocrit values ranged from 40 % to 65 % (median 56 %, n=56), the TRBC counts varied from 5.21×10^6/µl to 7.91×10^6/µl (median 6.49×10^6/µl, n=20) and TWBC counts ranged from 4.50×10^3/µl to 22.00×10^3/µl (median 8.00×10^3/µl, n=20). The MCV values were between 68.07 fl/cell and 102.00fl/cell (median 86.72 fl/cell, n=20). Immature erythrocytes accounted for 0.83-5.70% of all erythrocytes (median 2.86%, n=20). The only common blood parasite detected in adult birds was *Haemoproteus* sp. (24.7 % of samples, n=178). *Leucocytozoon* sp. was found in only one individual and seven birds were infected by avian filariae.

The description of leukocyte differential count in rosefinch nestlings is given in Table 2. In the nestlings, a range from 5.98% to 38.20% of all erythrocytes were classified as immature (median 22.59%, n=20). No blood parasites were detected in nestlings.

Clear differences exist between adults and nestlings in all parameters of leukocyte differential count ($n_{nestlings}$=155, n_{adults}=178, Wilcoxon rank-sum test for lymphocytes Z=-9.81, for heterophils Z=-8.62, for basophils Z=8.86 and for eosinophils Z=11.05, p in all cases

<<0.001) as well as in the percentage of immature red blood cells (Exact Wilcoxon rank-sum test for polychromatic cells $n_{nestlings}$=10, n_{adults}=10, W=148, p<0.001). Significant sex differences in adults were found only in some parameters of leukocyte differential count (Two-sample t-test for the eosinophil count after arcsin transformation t=2.83, n=178, p=0.005; see also H/L ratio in condition analysis) and in the representation of immature polychromatic erythrocytes among the red blood cells (Two-sample t-test t=2.11, n=20, p= 0.049). No sex differences were determined in the values for haematocrit, TWBC, TRBC or MCV.

Haematological parameters and condition

As most counts of individual leukocyte types are to some extent intercorrelated, we only analysed factors in association with H/L ratio (a commonly investigated parameter) and basophil count (a major leukocyte type in Scarlet rosefinch) between which the correlation was insignificant (Pearson's product-moment correlation t = -1.81, df = 161, p-value = 0.072, correl. coef.= -0.14). In adults the H/L ratio was significantly dependent on year (p<0.001), period of capture (p<0.001), individual sex (p=0.021), mass (p=0.003, in interaction with period of capture p=0.028) and size in interaction with sex (p=0.023). In contrast, the basophil count in adults did not vary significantly between years and was independent of sex or individual size. Basophil count was significantly associated with individual mass (predicting body condition, p=0.021, Fig. 1), period of capture (p<<0.001) and infection by blood parasites of the *Haemoproteus* genus (p=0.020). Minimal adequate models for H/L ratio and basophil count in adults are given in Tables 3 and 4.

We have found no significant minimal adequate model in the case of basophil count for nestlings (the model comprising interaction of hatching date and nestling mass was

insignificant as a whole, p=0.057, df=3, chi=7.52, n=151). However, the H/L ratio was significantly associated with nestlings size (p= 0.018) and mass (p=0.041; Table 5).

Although there was no interaction of immature erythrocyte count in adults with any condition indicator *per se*, we have found a significantly negative association between the percentage of immature erythrocytes and individual size (p=0.020, value ± SE = -1.417 ± 0.556, df = 1/18, F=6.49, n=20; Fig. 2). Values for haematocrit (n=56), TRBC (n=16), TWBC (n=16) and MCV (n=16) in adults were not correlated with individual size or mass (in all cases p>0.20). In nestlings, we have found a significantly positive association between immature erythrocyte count and growth (p=0.014, value ± SE = 15.943 ± 5.835, df = 1/18, F=7.47, n=20; Fig. 3).

Discussion

Our results have shown two interesting points: first, the basophil count may be an important parameter to assess in studies utilizing haematological methods; and second, immature cell count seems to serve as a useful predictor of growth and developmental speed in nestlings.

In the peripheral blood of Scarlet rosefinches we have found an unusually high proportion of basophils, reaching up to 86% of all leukocytes in adults and 91% in nestlings (medians 40% and 56%, respectively). Analysis of the association of leukocyte differential count with condition has revealed that, at least in adults, the basophil count serves as a better indicator of condition and health than the H/L ratio. While H/L ratio was dependent on factors that have no or only limited relationship to condition (such as year, sex or size), the basophil count was linked to individual mass (predicting condition) and *Haemoproteus* infection (indicating

individuals' health). In nestlings, however, not the basophil count but the H/L ratio correlated with condition-related traits (especially with individual mass).

To date, the function of basophils is only poorly understood in birds. This cell type seems to be involved in acute inflammatory defence and immediate hypersensitivity reactions (Daloia et al. 1994; Maxwell and Robertson 1995; Campbell and Ellis 2007). As avian basophil granules are extremely water soluble, they may be easily damaged during the staining process (Lucas and Jamroz 1961), which impairs the detection of these cells. This might be the reason why precise data on the variability of basophil levels in peripheral blood are limited in free-living birds (but see Davis 2009). Nevertheless, there is some clear evidence suggesting that the proportion of basophils among blood-borne leukocytes is much higher in some avian species than the normal physiological values of most mammals (Maxwell and Robertson 1995). A good example of natural variability in basophil counts was given by Friedl and Edler (2005), who found that the percentage of basophils ranges from 0% to 24% in the Red bishop (*Euplectes orix*). High levels of basophils in peripheral blood were also reported in some other passerine species (e.g. Pine siskin, *Carduelis pinus*, or Pied flycatcher, *Ficedual hypoleuca*; Davis 2009). Even in non-passerines such as Puna ibis (*Plegadis ridgewayi*; Coke et al. 2004), the Common pheasant (*Phasianus colchicus*; Lucas and Jamroz 1961), and some strains of the domestic chicken (Maxwell and Robertson 1995), normal basophil levels in adults may exceed 10%. Much lower basophil counts (0-5%) were detected in most finches, other than Scarlet rosefinches (Campbell and Ellis 2007; Davis 2009). Nevertheless, in the House finch (*Carpodacus mexicanus*), a species that is closely related to the Scarlet rosefinch, the basophil granulocytes are more frequent than heterophils in the peripheral blood of free-living individuals (Davis et al. 2004; Davis 2005). Thus our results are unusual due to the extreme number of basophiles but not inconsistent with the former findings.

Although basophils are known to be released into blood circulation in higher numbers due to stress (Maxwell 1993; Altan et al. 2003; Campbell and Ellis 2007; Bedáňová et al. 2007), it is unlikely that our results are caused by stressful manipulation of the birds. In the field, all blood samples were collected within 30 minutes after the capture and prior to any further manipulation of the individual. Davis (2005) previously reported that a handling time less than 1 hour does not influence the leukocyte differential count in peripheral blood of the House finch. Moreover, Scope et al. (2002) have shown that there are no significant changes in peripheral blood basophil numbers even 3 hours after the stressful event. Acute stress is known to increase the H/L ratio (Lazarevic et al. 2000; Ewenson et al. 2001; Ruiz et al. 2002; Scope et al. 2002; El Lethey et al. 2003; Bedáňová et al. 2007; Davis et al. 2008) and we did not find unusually high proportions of heterophils in our smears. Furthermore, a high proportion of basophils was similarly recorded in blood from nestlings for which no potentially stressful capturing was performed. We propose that the high levels of basophils are present in this passerine species with long-distance migration as a part of a particular immunological anti-parasite defence. Levels of basophils are known to reflect infection status of some diseases (Falcone et al. 2001). Also our results show higher basophil levels in birds suffering from *Haemoproteus* infection and those in worse condition (having a lower body mass). This is in concordance with findings of earlier studies reporting that *Haemoproteus* infection alters the health state as well as blood parameters (Ots and Hõrak 1998) including basophil levels (Garvin et al. 2003).

In our study we have shown that the immature erythrocytes are reasonably common in Scarlet rosefinch peripheral blood circulation (in nestlings much more than in adults). Even in our limited samples we were able to detect clear association between the immature erythrocyte

count and size in adults and with growth rate in nestlings. These results suggest that immature erythrocyte count may represent an important haematological trait related to individual development and energetic costs.

A large body of convincing evidence from both birds and mammals suggests that a high percentage of immature erythrocytes among the red blood cells is indicative of disease, toxicosis or anaemia (Constantino and Cogionis 2000; Campbell and Ellis 2007). Still, the occurrence of small quantities of immature erythrocytes in peripheral blood is a normal state in birds (Campbell and Ellis 2007). In this study, we observed no clinical signs of illness among the individuals investigated. All birds had healthy appearance and their locomotive activity was normal. The values for haematocrit and the percentage of polychromatic erythrocytes among the red blood cells obtained in our sample of adults also did not indicate any abnormality when compared to the values published for healthy captive-held passerines (Campbell and Ellis 2007). On the other hand, both TRBC and TWBC had higher levels than those described for captive-held passerines. We assume that high TRBC and perhaps also TWBC levels may represent ecological adaptation in this species. Greater amounts of erythrocytes may be required during the long distance migration to support the high oxygen demands of the tissues under intense physical effort. Although the numbers of leukocytes in our sample had a much greater range than the values known for finches bread in captivity, in most cases our values lie within the normal values published. The sample was, unfortunately, too small to try to explain the reason for elevated leukocyte levels in some of the individuals. Nonetheless, as the high levels of immature erythrocytes in peripheral blood indicate the presence of blood-regenerative processes (Yamato et al. 1996; Campbell and Ellis 2007; Carleton 2008), we hypothesise that this trait may be related to the rate of blood cell formation and thus possibly also to metabolic rate in healthy individuals.

Conclusion

This study has clearly highlighted the necessity of the basic examination of the cellular composition of peripheral blood prior to simplification of the health-evaluating method. Contrary to most mammals and poultry, some avian species may circulate in their peripheral blood relatively high proportions of leukocytes of other types than lymphocytes and heterophils, e.g. basophils. Researchers in ecology should be aware of this fact. We suggest that basophil count may represent a valuable indicator of health in some species. We do not recommend blood smear staining solely with Giemsa's staining solution (e.g. suitable for parasite detection) even in the cases with high proportions of heterophils in the blood films. This method severely impairs the discrimination of leukocytes, which makes the results less reliable. Moreover, when differential staining is adopted, the erythrocyte differential count may be also estimated. Our results indicate that immature erythrocyte count may serve as a suitable tool for estimating development. In avian haematology we also advise forgoing fixation with methanol prior staining. Methanol fixation decreases the visibility of granules in basophils and thus worsens their detection (Robertson and Maxwell 1990; basophils without clearly visible granules can resemble lymphocytes and thus bias even the resulting H/L ratio). However, notwithstanding these potential methodological pitfalls, if appropriate technique is adopted, haematological methods represent a useful and reliable tool for the estimation of health status.

Zusammenfassung

Hämatologische Untersuchung des Gesundheitszustands in Singvögeln mit extrem hohem Anteil basophiler Blutkörperchen in peripherem Blut

Hämatologische Methoden werden von Ökologen häufig für die Untersuchung des individuellen Gesundheitszustandes von Vögeln verwandt. Die technische Einfachheit einiger dieser Tests kann jedoch zu einer starken Vereinfachung der Evaluation derselben führen. Hier zeigen wir an Karmingimpeln (*Carpodacus erythrinus*) die Bedeutsamkeit anderer hämatologischer Parameter als das häufig genutzte Verhältnis Heterophiler Zellen zu Lymphozyten (H/L ratio). Wir beschreiben sieben hämatologische Charakteristika im Detail (Leukozyten Differentialblutbild, unreife Erythrozytenzahl, Hämatokrit, das mittlere Zellvolumen, die Gesamtzahl der roten und weißen Blutkörperchen und das Auftreten von Blutparasiten). Besonders aufgefallen bei der Untersuchung von 178 Adulten und 155 Nestlingen war ein außergewöhnlich hoher Anteil Basophiler im peripheren Blut. Obwohl der große Anteil Basophiler ein generelles Charakteristikum auch in gesunden Individuen dieser Art ist, war der Anteil dieser Zellen abhängig von der Kondition und nahm bei Infektion durch *Haemoproteus* zusätzlich zu. Unsere Ergebnisse lassen auch vermuten, dass die Anzahl immaturer Erythrozyten im peripheren Blut ein guter Indikator für die Wachstumsrate von Nestlingen ist. Wir schlussfolgern, dass das Blutbild von Kardinalgimplen auffällig anders ist als das von anderen Vogelarten mit bekannten Baseline-Werten hämatologischer Parameter. Deshalb betonen wir die Notwendigkeit hämatologischer Voruntersuchungen, gründend auf Daten erfasst mit einer angemessenen Methode (z. B. für Blutausstriche empfehlen wir unterschiedliche Färbungen und die Vermeidung von vorhergehender Fixation durch Methanol).

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Tables

Table 1: Leukocyte differential count in scarlet rosefinch adults (n=178).

Cell type	Range (%)	Mean (%) ± SD	Median (%)
Lymphocytes	3.60 - 73.39	28.28 ± 13.04	25.67
Immature leukocytes	0.00 - 5.50	0.67 ± 0.99	0.00
Heterophils	0.00 - 71.88	18.73 ± 11.85	17.67
Basophils	13.33 - 85.60	41.93 ± 14.89	39.60
Eosinophils	0.00 - 27.82	6.60 ± 5.43	5.42
Monocytes	0.00 - 15.13	3.78 ± 2.99	3.25

Table 2: Leukocyte differential count in Scarlet rosefinch nestlings (n=155).

Cell type	Range (%)	Mean (%) ± SD	Median (%)
Lymphocytes	4.59 - 41.38	15.49 ± 6.63	14.17
Immature leukocytes	0.00 - 18.58	2.13 ± 2.50	1.59
Heterophils	0.00 - 25.86	9.12 ± 4.49	8.53
Basophils	7.76 - 90.99	55.86 ± 10.80	55.73
Eosinophils	0.83 - 33.88	15.48 ± 7.18	14.63
Monocytes	0.00 - 10.74	1.92 ± 1.90	1.57

Table 3: Minimal adequate model for H/L ratio in adults, n=163, df=10/162, F=4.62, p<<0.001. Slope values are given only for continuous variables.

Variable	Slope ± SE	df	F	p
Year		4/152	5.28	<0.001
Sex		2/152	3.96	0.021
Period of capture		2/152	8.84	<0.001
Size	-0.085 ± 0.040	2/152	2.89	0.058
Mass	2.081 ± 0.375	2/152	5.98	0.003
Sex : size	0.252 ± 0.037	1/152	5.29	0.023
Period of capture : mass	2.453 ± 0.378	1/152	4.95	0.028

Table 4: Minimal adequate model for basophil count in adults, n=163, df=3/159, F=9.30, p<<0.001.

Variable	Slope ± SE	df	F	p
Mass	-0.427 ± 0.183	1/159	5.42	0.021
Period of capture	-0.049 ± 0.012	1/159	16.87	<<0.001
Haemoproteus inf.	0.031 ± 0.013	1/159	5.49	0.020

Table 5: Minimal adequate model for H/L ratio in nestlings, n=151 nestlings in 43 nests, df=2, Chi=15.89, p<0.001. Model is based on GLMM modelling with nests treated as random effect.

Variable	Slope ± SE	df	Chi	p
Size	-0.133 ± 0.051	1	5.62	0.018
Mass	-1.189 ± 0.581	1	4.16	0.041

Figures

Fig. 1: The association of individual mass with basophil count in adult Scarlet rosefinches (n=163). Differential basophil count is given as residuals of the arcsin transformed values after controlling for period of capture and *Haemoproteus* infection (for the minimal adequate model see Table 4); mass (individual weight divided by tarsus length) is given in g/mm.

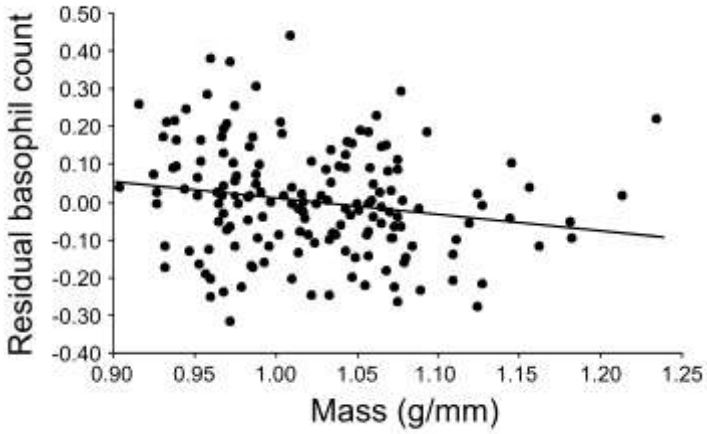

Fig. 2: Association of the immature erythrocyte percentage and individual size in adult Scarlet rosefinches (n=20). For details to the immature erythrocyte percentage see methods; size is given as individual tarsus length in mm.

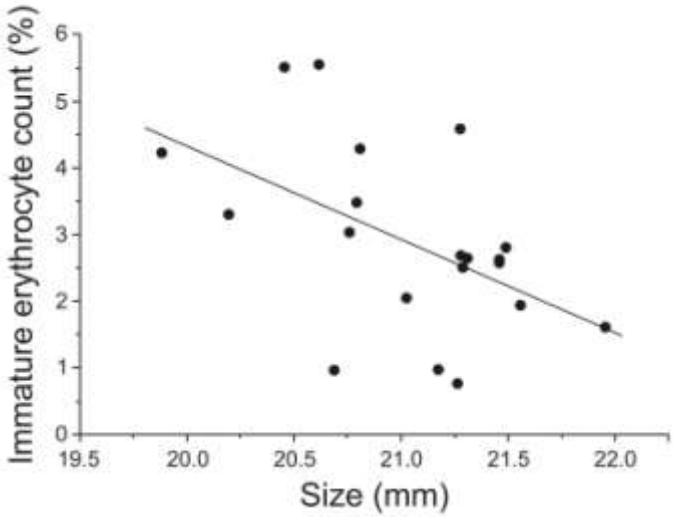
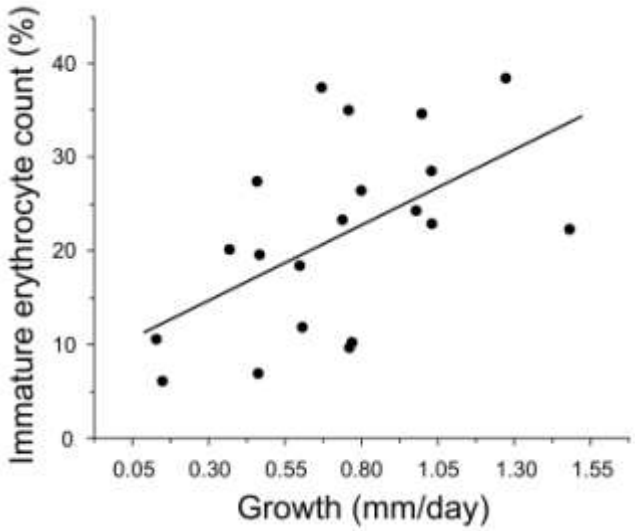
Fig. 3: Association of immature erythrocyte count and growth in Scarlet rosefinch nestlings (n=20). For details to the immature erythrocyte percentage see methods; nestling growth is given as individual tarsus length in mm on day 8 post hatch minus the length on day 7 post hatch.