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Effects of different environmental and sampling variables on the genotyping success in field-collected scat samples: a brown bear case study

Vpliv različnih dejavnikov okolja in vzorčenja na uspešnost genotipizacije vzorcev iztrebkov, zbranih na terenu: primer pri rjavem medvedu

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Abstract: The paper investigates how different field conditions and sample characteristics influence genotyping success in field-collected brown bear scat samples. Genotyping performance of 413 samples collected in a pilot study in southern Slovenia was evaluated, and statistical modelling was used to control confounding between predictor variables and to quantify their specific effects on genotyping success. The best predictors of genotyping success were subjectively estimated scat age, sampling month, and contents of a scat. Even when the other confounded variables were controlled for, genotyping success dropped rapidly with the age estimate, from 89% (82-94%) for 0-day scats to 33% (19-52%) for scats estimated to be 5 days old. Sampling month was also an important predictor, and samples collected during the bear hyperphagia period in late summer / autumn performed considerably better (90%, 78-96%) than the samples collected in spring / early summer (66%, 57-74%). This effect was stronger for fresh than for older samples. Effects of different food types were also considerable, but less important for practical use. Since noninvasive genetic sampling already became the key method for surveying wild populations of many species, efficiency of studies is becoming increasingly important. Understanding the effect of the month of sampling allows the field season to be timed for maximum genotyping success, while subjective scat age provides a useful metric that indicates a sample's viability for genotyping, allowing for prioritization of samples and culling of non-viable samples before resources are wasted for their analysis. This provides higher useful data yields per invested resources and may ultimately lead to better study results.

Keywords: genetics, genotyping success, molecular ecology, noninvasive sampling, scat sampling, *Ursus arctos*

Izvleček: V članku je predstavljen učinek različnih terenskih pogojev in lastnosti vzorca na uspešnost genotipizacije iztrebkov rjavega medveda, nabranih na terenu. Ocenil sem uspešnost genotipizacije 413 vzorcev, zbranih v pilotni študiji v južni Sloveniji ter uporabil statistično modeliranje za popravek motenja med spremenljivkami in kvantifikacijo njihovih učinkov na uspeh genotipizacije. Uspeh genotipizacije so najbolje pojasnili subjektivno ocenjena starost vzorca, mesec vzorčenja in vsebina iztrebka. Tudi ko sem kontroliral moteče spremenljivke, je uspešnost z višjo oceno starosti hitro padala, od 89 % (82 – 94 %) pri iztrebkih starih 0 dni na 33 % (19 – 52 %)

za iztrebke ocenjene kot stare 5 dni. Pomembna pojasnjevalna spremenljivka je tudi mesec vzorčenja, saj so imeli iztrebki, zbrani v obdobju hiperfagije medvedov pozno poleti in jeseni znatno višjo uspešnost (90 %, 78 – 96 %) kot iztrebki zbrani pomladi in zgodaj poleti (66 %, 78 – 96 %). Ta učinek je bil izrazitejši za sveže kot za starejše vzorce. Učinki različne prehrane so bili prav tako precejšnji, kar pa je za praktično uporabo manjšega pomena. Neinvazivno genetsko vzorčenje je že postalo ključna metoda za preučevanje prostoživečih populacij številnih živalskih vrst, zato postaja učinkovitost takšnih študij vse bolj pomembna. Razumevanje učinka meseca vzorčenja nam omogoča načrtovanje terenskega dela tako, da bo uspešnost genotipizacije kar najvišja. Po drugi strani nam subjektivna ocena starosti iztrebka podaja dobro merilo uporabnosti vzorca in nam omogoča prioritizacijo vzorcev ter odstranitev slabih vzorcev, preden porabimo sredstva za njihovo analizo. To omogoča višji izplen uporabnih podatkov glede na porabljena sredstva in delo ter lahko prispeva k boljšim rezultatom študije.

Ključne besede: genetika, molekularna ekologija, neinvazivno vzorčenje, *Ursus arctos*, uspešnost genotipizacije, vzorčenje iztrebkov

Introduction

Noninvasive (or minimally invasive) genetic sampling is increasingly becoming the key method for surveying wild populations of many species (DeYoung and Honeycutt 2005, Carroll et al. 2018). It allows us to collect large numbers of genetic samples in a cost-effective manner without disturbing the animals or affecting their behavior. Consequently, it is being used for a wide range of research and management questions such as population genetics, breeding behavior, population abundance, and breeding behavior (DeWoody 2005, Waits and Paetkau 2005, Mumma et al. 2016, Carroll et al. 2018). New applications and new analytical approaches emerge regularly as researchers develop new ideas on how to use these powerful tools (De Barba et al. 2017, Andrews et al. 2018, Carroll et al. 2018).

There is also a downside to using noninvasive genetic samples. Exposed to harsh field conditions, DNA in such samples rapidly deteriorates, decreasing genotyping success and generating errors that must be correctly handled in laboratory analysis and downstream analytical procedures (Taberlet et al. 1996, 1999). Consequently, development of noninvasive genetic sampling has not been without its growing pains, and serious errors were made by researchers that were not sufficiently aware of the specifics of data obtained from noninvasive genetic samples (e.g. Gagneux et al. 1997, retracted 2001). Nevertheless, the methods matured over the last two decades into routinely applicable tools which can be, with some care, applied by any competent researcher for a wide range of research questions.

Researchers have dedicated considerable effort to improve the laboratory procedures and maximize genotyping success (e.g. De Barba and Waits 2010, Skrbinšek et al. 2010). Regardless of the well understood analytical approaches for noninvasive genetic samples, one critical issue remains: DNA quality. As a rule, not all noninvasive genetic samples collected in the field will provide useful data, and in some cases the success rate can be quite low (Waits and Paetkau 2005). A non-amplifying sample still requires effort and generates costs. A large proportion of failed samples increases the cost and effort, even in well-planned studies that prepare for that contingency, and can even cause studies to fail in meeting their goals if such contingency planning is insufficient. A firm understanding of when and how to plan the field season to maximize genotyping success rate, which field conditions can influence that parameter and how to decide whether or not a sample is viable for analysis are critical factors that can help a researcher design a more efficient study that can provide more useful data per given field effort and costs. While there are several published experimental studies where some specific field factors affecting genotyping success in noninvasive samples have been looked at (e.g. Murphy et al. 2007, Santini et al. 2007), there are not many studies that would specifically

try to examine field-collected samples post-hoc and thoroughly evaluate how they performed with regard to the sampling conditions, appearance and content (but see Kopatz et al. 2020).

In this paper, I explore how different field conditions and sample characteristics affect genotyping success, the proportion of samples that can be successfully genotyped, in field-collected brown bear scat samples. I use statistical modelling to correct for confounding between environmental variables and scat appearance to evaluate specific effects of individual variables on genotyping success. Finally, I summarize the findings into recommendations that can be used to increase fieldwork efficiency in studies utilizing scat samples and provide suggestions for further research of the topic.

Materials and methods

Data collection and laboratory analysis protocols are described in details in Skrbinšek et al. (2010). Here I use 413 samples collected in a pilot study in two predefined sampling areas (170 and 240 km^2, respectively) between May and November of 2004 and 2005. The samples were collected by volunteer samplers and by professional hunters of Slovenia Forest Service. Both areas are in Dinaric Mountains of southern Slovenia, (area 1: 45.68328N, 14.67084E; area 2: 45.61652N, 14.43271E).

Each person involved in sampling (sampler) received an oral presentation about the sampling procedures and precise written instructions for sample collection, evaluation of its age, and recording of other environmental parameters. Samplers were instructed to collect the sample from the outer layer of the scat, possibly a part not in contact with the floor, and not from the top of the scat where the DNA is most exposed to being washed away by rain. Samples were collected in 50 ml screw-cap tubes in non-denatured 96% ethanol. Upon delivery to the lab the samples were stored at -20°C until analysis.

The length of the period that the scat has been exposed to the elements effects the quality of the target DNA (Murphy et al. 2007, Panasci et al. 2011), so we provided the samplers with guidelines how to estimate the scat's age. These guidelines were not precise, but rather helpful pointers to distinguish old scats from fresh ones (contents specific smell, visual appearance, presence of mucous and insect larvae). We also instructed the samplers not to collect samples they judge to be older than 5 days, thus providing an upper subjective 'limit' on what they considered to be a fresh enough scat for analysis. The samplers also collected additional data about the sample – date and location where it was found, and how protected it was by foliage (exposure). During DNA extraction the main contents of each scat were recorded, and later organized into five broad categories: green vegetation, material of animal origin, beech nuts, corn, and fruits.

Laboratory analysis is explained in detail in Skrbinšek et al. (2010). Briefly, DNA from the samples was extracted in a dedicated laboratory for noninvasive genetic samples with very strict contamination prevention protocols. DNA template was amplified using 12 microsatellite markers and a sex marker in a single multiplex PCR following the modified multiple-tube approach (Taberlet et al. 1996, Skrbinšek et al. 2010). Fragment analysis was performed on an Applied Biosystems ABI 3130xl automatic sequencer, and data analyzed using GeneMapper 4.0 software (Applied Biosystems) to provide single-PCR genotypes. The genotype data from multiple amplifications for each sample were pooled to obtain consensus genotypes and calculate the quality index (Miquel et al. 2006b), the average proportion of genotyping analyses that provided the correct (consensus) genotype.

We were able to successfully genotype many poor-quality samples because they matched a good-quality sample, but which we would not be able to reliably genotype on their own. To get a sample-level objective criterion, I used the quality index (QI) as a measure of DNA quality rather than the actual information if we were able to successfully genotype the sample or not. Since this metric was distributing bimodally (Fig. 1), I recoded it into a binary variable 'genotyping success', with samples that had QI < 0.4 considered as 'failed'. This threshold was obtained through experience and was used also in the original study as the 'fail' threshold for samples that didn't match any other sample of the same animal (Skrbinšek et al. 2010).

I obtained meteorological data (temperature, precipitation) from automatic meteorological stations in the area. For each sample I extracted the data from the closest meteorological station for the time period it was expected to be exposed in the environment – since it was presumably deposited by the animal (estimated using scat age estimate) until it was collected (recorded date). Total precipitation and average temperature during that period were calculated for each sample. Temperature was corrected for difference in elevation between the meteorological station and the location where the sample was collected.

I explored the effects of the sample and environmental parameters on genotyping success using Generalized Linear Models (GLM) and information-theoretic analysis approach (Burnham and Anderson 2002). All analyses were done in R language for statistical computing (R Core Team 2020) using RStudio IDE version 1.3.1073 (R Studio Team 2020). I used 'genotyping success' as the response variable with the binomial link function, and constructed an a-priori model set that reflected the following hypotheses: a) the DNA in scat degrades with its age - the time it is exposed in the environment, b) an increase in environment temperature can affect the DNA quality in the scat either by conservation through dehydration, or increased decomposition due to higher enzymatic and bacterial activity, c) rain can wash target DNA from the scat sample, d) scat contents directly affects the quantity and quality of target DNA through "scraping" of intestinal epithelium by rough food particles and conservation of DNA in the environment but can also negatively affect genotyping success because of PCR inhibiting substances, e) food and climate conditions change through the cycle of seasons and can have complex effects on analytical viability of target DNA, f) content of the scat possibly exacerbates or ameliorates the effect of age, temperature, sampling month and rain, and g) age of the scat can have different effects in different months because of different climatic and food conditions.

I first fitted the full model of all variables that I hypothesized could affect the genotyping success, without interactions, and explored Variance Inflation Factors (VIF) to check for multicollinearity. I fitted the models that reflected the hypotheses stated above, with the limitation that the maximum number of estimated parameters in each included model didn't exceed 12 to avoid overparameterization and used AICc as the model selection criterion (Akaike 1973). Since there is an obvious relation between variables 'month of sampling' and 'temperature', I didn't use both variables in the same models. I used the most parsimonious model (with the lowest AICc) as the best model for inference, but I also performed averaging of models within dAICc <= 5 using Akaike's weights (Burnham and Anderson 2002) to explore model selection uncertainty and the effects of parameters and interactions that were not included in the best model. I used R packages ggeffects (Lüdecke 2018) and ggplot2 (Wickham 2016) to explore and visualize the effects of different explanatory variables on the response variable and package MuMIn (Bartoń 2020) for calculating AICc and model averaging.

Results

Analysis of Variance Inflation Factors (VIFs) didn't indicate any problematic multicollinearity between variables (generalized VIFs between 1.02 and 1.67).

Models that included the 'month of sampling' variable were considerably better than the models that included 'temperature' (dAICc = 5.43 between best models), and the 'temperature' variable was not considered any longer. All models within dAICc < 3 included scat age, month of sampling and scat content variables. Interaction between age and month of sampling was also represented in many high-ranking models (Akaike weight sum = 0.69). These variables were included in the highest-ranking (best) model, which was used for inference, and their distribution is shown in Fig. 1. Use of the other variables had much less support in the data and were not considered anymore.

Observed effects of the variables predicted using the best model were compared with model predictions obtained with the model-averaged model. The differences were small, and the averaged model was not considered anymore.

Estimated age of the scat had the most prominent effect on genotyping success (Fig. 2A). When controlled for the other parameters (consecutive day in a year (sampling month variable) = 222 (average),

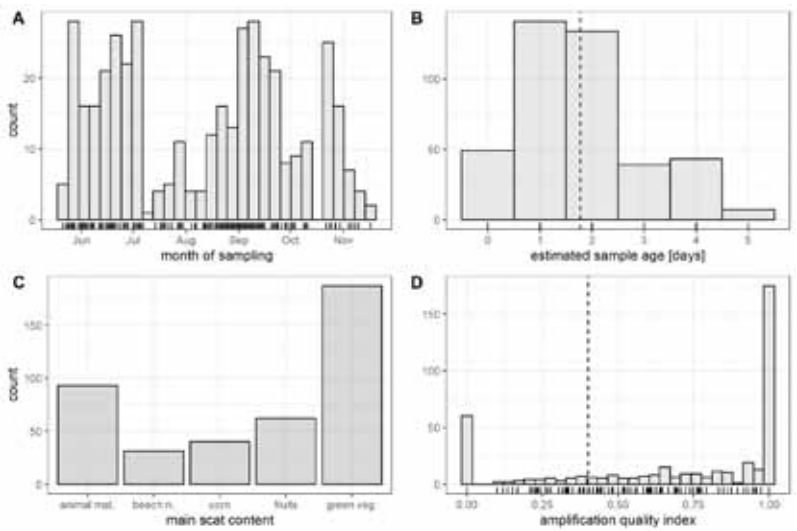

Figure 1: Distribution of samples by month of sampling (**A**), subjectively estimated sample age (**B**), the main visually estimated scat content (**C**), and amplification quality index (Miquel et al. 2006b) (**D**). The dashed vertical line in (B) indicates the average estimated age. The dashed vertical line in (D) indicates the 0.4 quality index threshold above which the sample was considered suitable for genotyping.

Slika 1: Razporeditev vzorcev po mesecu vzorčenja (**A**), subjektivni oceni starosti vzorca (**B**), glavni vizualno ocenjeni vsebini iztrebka (**C**) in indeksu kvalitete (Miquel et al. 2006a) (**D**). Črtkana navpična črta v (B) prikazuje povprečno ocenjeno starost vzorca. Črtkana navpična črta v (D) prikazuje prag indeksa kvalitete 0.4, nad katerim se vzorec smatra kot ustrezen za genotipizacijo.

content = 'green vegetation' (most frequent)), the expected success rate for samples estimated to be 0 days old was 89% (82-94%). This expectation drops to 33% (19-52%) for the samples estimated to be 5 days old.

The sampling month was another important predictor of genotyping success (Fig. 2B), with one and two days old samples (scat age = 1.5, content = 'green vegetation') collected in spring and early summer having a considerably lower expected success rate (66%; 57-74% CI) than similar samples collected in late autumn (90%; 78-96% CI). The interaction between age of the scat and the sampling month indicates that this effect is stronger for fresher samples and disappears for samples estimated to be 4 and 5 days old (Fig. 2D). However, the confidence intervals, which are relatively narrow for fresh samples, are much wider for older samples (Fig. 2D) since there were considerably more fresh samples collected (Fig. 1B), so the actual effect for older samples is less clear.

Scat content was also an important predictor of genotyping success (Fig. 2C), with beech nuts having considerably higher expected success rate, followed by corn. However, it must be noted that beechnuts are available only in autumn and early winter when success rate is generally higher. Most of the samples with fruit content were also sampled in autumn.

Discussion

In this study, I explored a number of field-collected variables about environmental conditions, time of the year and visual appearance of scat samples collected for genotyping. Using statistical modelling, I was able to disentangle the effects of otherwise confounded variables and found some of them to be useful predictors of genotyping success. Understanding these effects can help researchers in both fieldwork planning

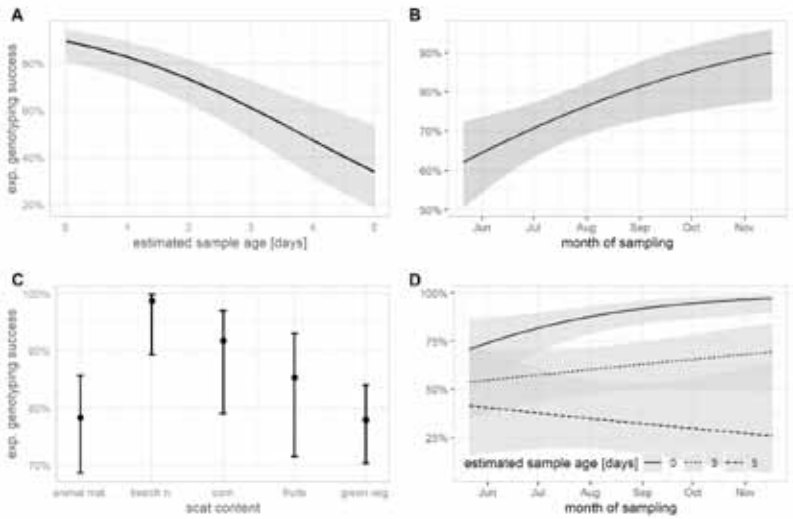

Figure 2: Model-predicted expected genotyping success by estimated sample age (**A**), month of sampling (**B**), main content of scat (**C**) and month of sampling by estimated sample age (**D**). For calculations of predictions, all other parameters were set to the average value (for continuous variables) or set to the most frequent value (for categorical variables). Gray ribbons in A, B and D and bars in graph C indicate 95% confidence intervals of predictions. Graph D doesn't show all values for sample age to be able to show the confidence intervals. None of the graphs were extrapolated outside of the collected data.

Slika 2: Modelne napovedi pričakovane uspešnosti genotipizacije glede na ocenjeno starost vzorca (**A**), mesec vzorčenja (**B**), glavno vsebino v iztrebku (**C**) in mesecu vzorčenje glede na ocenjeno starost vzorca (**D**). V vseh primerih so bile za izračun napovedi vse ostale spremenljivke nastavljene na aritmetično sredino (pri zveznih spremenljivkah) oziroma na najbolj pogosto vrednost (pri kategoričnih spremenljivkah). Siv pas v A, B in D ter navpične črte v C kažejo 95% intervale zaupanja. Graf D ne prikazuje vseh vrednosti ocenjene starosti vzorca, da se lahko prikažejo intervali zaupanja. Grafov nisem ekstrapoliral izven okvirjev zbranih podatkov.

and laboratory analysis, potentially increasing the amount of useful data collected in a study and reducing costs.

A failed analysis of a noninvasive genetic sample means that all (usually considerable) resources that went into its collection and laboratory analysis have been wasted. Consequently, one of the major targets during implementation of any study utilizing such material is to optimize both the sample collection and the laboratory analysis in a manner that maximizes the genotyping success, providing the maximum yield of useful data for the invested effort and funds. An understanding of the field-observable variables that influence the genotyping success can help in fieldwork planning and provide field personnel with useful information on which samples to collect and what data to record. A good understanding of the expected genotyping success rate also helps in scaling of the field effort for maximum efficiency – to avoid wasting resources through oversampling, but to still provide enough sample coverage to obtain the desired result. When these samples are being analyzed in a laboratory, we can use the success rate predictions to prioritize samples, which is particularly useful if more samples have been collected than there are resources available to analyze them.

One of the major observations is that a subjective estimate of scat age is an excellent predictor of the scat's amplification success. The fact that target DNA degrades with time in a noninvasive genetic sample is intuitive, and has been demonstrated experimentally by several authors (Murphy et al. 2007, Santini et al. 2007, Panasci et al. 2011, Demay et al. 2013), with most authors

describing rapid degradation of DNA in the first 5-7 days (but see King et al. 2018). The interesting observation of my work is that a person in the field, equipped just with basic instructions, their senses, and common sense, does an excellent job in estimating freshness of a scat. Scats will deteriorate at a different pace depending on many factors, environmental and intrinsic, most of them difficult or impossible to evaluate in the field. This means that a subjective estimate of a scat's "age" will not necessarily reflect the exact period it spent being exposed in the environment, but rather the sum effects of both the time, the scat's composition, and the environmental factors that impacted its subjective appearance of "freshness". However, the high predictive power of this subjective estimate suggests that it is possibly more useful than knowing the objective age of a scat would be.

Another interesting finding is the considerable effect of the month of sampling. A similar effect of season on genotyping success from feces has been described before (Piggott and Taylor 2003, De Barba et al. 2010, Kopatz et al. 2020). Interestingly, the Kopatz et al. (2020) study done recently on brown bear scats collected in northern Norway as well as De Barba et al. (2010) study done in Italian Alps both found almost the same effect, in the same species, but in very different landscapes. It is difficult to determine what is causing this effect, but we can speculate on some interactions of climate and food conditions. An interesting speculation is also that this higher success may be caused by the brown bear seasonal physiological cycle since the higher genotyping success coincided with the late summer / autumn hyperphagia period (López-Alfaro et al. 2013), both in our study and in the study done in northern Norway. The interaction between the month of sampling and the estimated age of a sample also indicates that the month of sampling had a stronger effect on fresh samples. While there is a decent sample coverage for most of the period when brown bear scat sampling is typically feasible in our climate, the coverage is lower from mid-July until mid-August, making predictions for that period less reliable.

The effect of the scat content (diet) has been observed to influence genotyping success of scat samples (Murphy et al. 2003, Broquet et al. 2007, Panasci et al. 2011), but it is more difficult to interpret than other variables, and of less practical use. Beech nuts, one of the main bear foods in autumn during high beech mast years, has extremely high success rate, but this finding may be biased by scat age estimates. Scats with high beech nut content may look old very quickly (personal observation), and there is a fair chance that the samplers were overestimating the age of the scat, or even skipped collecting some relatively fresh scats they deemed too old (i.e. subjectively estimated to be older than five days).

Even if I have not found support for the effects of the other parameters I considered, this does not mean that they do not exist. Rainfall and exposure were recognized as a considerable factor of DNA degradation by other authors (Murphy et al. 2007, Brinkman et al. 2010), but didn't show in any of the high-support models in this study. The reason for this may be that we didn't collect scats that were subjectively considered older than 5 days, and high rainfall on an exposed scat could have considerably altered a scat's appearance, making it look older than it was and hence not considered for collection. For environmental temperature I did observe an effect, but as this variable is highly correlated with the sampling month, I didn't use both variables in the same model, and use of the sampling month was much better supported.

Conclusions

While not all the results presented here will be applicable to other geographic locations and/or species, there are concrete practical applications of the findings described in this paper.

A surprisingly good predictor of genotyping success, the subjectively estimated age of the scat should be recorded in any noninvasive genetic study. While it is safe to assume that its predictive power would be considerable in any study of brown bears, it should also be a reasonably good predictor in other species where scat samples are used to obtain genotypes. In the latter case, caution should be exercised since it does seem that in certain species and environments the DNA degradation process in scat can be much slower (King et al. 2018). But with some preliminary research into the effect of this variable on genotyping success, it can be effectively used

both during the field collection as well as in the lab to select and prioritize samples for analysis, increasing effectiveness of the study and decreasing costs.

The effect of the month of sampling seems like another very important predictor of genotyping success, and one that can have a considerable impact on study design since a researcher can considerably increase the effectiveness of a study just by correctly planning the sampling season. The fact that we can see nearly identical effects of the month of sampling in southern and northern Europe indicates that at least for brown bears in Europe, we may be able to generalize this observation. The data I show here come from a pilot study, and when the effect of the sampling month became evident in our preliminary analyses, we timed our country-wide sampling of brown bears from September until December, and achieved 88% genotyping success (Skrbinšek et al. 2019). While seasonal effects will almost certainly be different in other species and landscapes (e.g. Piggott 2004), a thought should be given to this issue during the planning phase of any study utilizing noninvasive genetic samples. A well-conceived pilot study that investigates this may end up saving a lot of effort and costs in the long run.

As noninvasive genetic sampling slips from the domain of cutting-edge science into a more mundane domain of routine everyday use, optimization and cost-effectiveness increasingly become the critical issues. For many studies, understanding the factors that improve or deteriorate genotyping success may just mean the difference between a study succeeding, or failing miserably.

Povzetek

Neinvazivno genetsko vzorčenje je postalo ključna metoda za preučevanje prostoživečih populacij različnih živalskih vrst, saj nam omogoča cenovno učinkovito zbiranje velikega števila genetskih vzorcev, ne da bi s tem živali motili ali vplivali na njihovo vedenje. So pa takšni vzorci z analitičnega vidika zahtevni, uspešnost analize (genotipizacije) pa je lahko v posameznih študijah zelo nizka. Zaradi tega je pomembno poznati dejavnike, ki vplivajo na uspešnost analiz, saj nam lahko to pomaga pri načrtovanju in laboratorijskih analizah, kar lahko prispeva k učinkovitejši in uspešnejši študiji.

V članku sem ocenil vplive različnih okoljskih dejavnikov in zabeleženih lastnosti vzorcev na uspešnost genotipizacije 413 vzorcev, zbranih v pilotni študiji v južni Sloveniji. Ker je spremenljivk veliko in ker je med njimi precej motenja, sem uporabil statistično modeliranje z generaliziranimi linearnimi modeli in informacijsko-teoretični pristop izbire modelov za prepoznavo najpomembnejših pojasnjevalnih spremenljivk, popravek motenja med njimi in kvantifikacijo njihovih učinkov.

Uspeh genotipizacije so najbolje pojasnili subjektivno ocenjena starost vzorca, mesec vzorčenja in vsebina iztrebka. Tudi ko sem kontroliral moteče spremenljivke, je uspešnost z višjo oceno starosti hitro padala, od 89 % (82 – 94 %) pri iztrebkih starih 0 dni na 33 % (19 – 52 %) za iztrebke ocenjene kot stare 5 dni. Pomembna pojasnjevalna spremenljivka je bil tudi mesec vzorčenja, saj so imeli iztrebki, zbrani v obdobju hiperfagije medvedov pozno poleti in jeseni znatno višjo uspešnost (90 %, 78 – 96 %) kot iztrebki zbrani pomladi in zgodaj poleti (66 %, 78 – 96 %). Ta učinek je bil izrazitejši za sveže kot za starejše vzorce. Učinki različne prehrane so se zdeli prav tako precejšnji, vzorci z visoko vsebnostjo žira ali koruze pa so se izkazali kot najboljši. Za praktično uporabo je sicer ta spremenljivka manjšega pomena, zaradi sezonske dostopnosti posamezne hrane pa tudi motenja nisem mogel v celoti kontrolirati.

Neinvazivno genetsko vzorčenje vse bolj prehaja iz domene vrhunske znanosti v domeno rutinske vsakodnevne uporabe, kar še bolj poveča potrebo po čim višji učinkovitosti študij. Razumevanje učinka meseca vzorčenja nam omogoča načrtovanje terenskega dela tako, da bo uspešnost genotipizacije kar najvišja. Po drugi strani nam subjektivna ocena starosti iztrebka podaja dobro merilo uporabnosti vzorca in nam omogoča prioritizacijo vzorcev ter odstranitev slabih vzorcev, preden porabimo sredstva za njihovo analizo. To omogoča višji izplen uporabnih podatkov glede na porabljena sredstva in delo ter izboljša uspešnost študije, pri nekaterih študijah pa lahko celo pretehta, ali bo študija uspela ali ne.

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