

## **Effect of the roads on small mammal populations**

By  
**Adriana RICO**

Faculty of Biological Sciences, university of South Bohemia,  
Ceske Budejovice Czech Republic

In this thesis, the author evaluated the negative effects of roads and highways on dispersal movements of three species of small mammals (two rodents and one shrew). The possible barrier effect of narrow, intermediate and wide roads is tested. The thesis has four parts, an introduction followed by three papers. The first part introduces the objectives of the work and provides some general information on the two most abundant species (the bank vole and the yellow-necked mouse). The results are detailed in three papers following the introduction. The two first papers are already accepted for publication in the journals *Folia Zoologica* and *Acta Theriologica* and the third one is currently submitted to *Molecular Ecology* and still under revision.

The first paper aims to determine to which extent roads act as barriers for the three species and what is the relative importance of road width and traffic intensity on the barrier effect. The main results indicate that roads prevent crossing movements of all three species although there are interspecific differences in crossing rates. Species cross more often narrow than wide roads but traffic intensity does not affect the crossing rates.

I think data analysis is well done with appropriate statistical methods and conclusions are closely supported by results as well. Nevertheless I have some remarks and questions about the sampling design.

The two trap lines on each side of the roads were distant of only 5 meters which is a very short distance considering mean spontaneous movements of these small mammals species. This may lead to a greater capture probability on the same side of capture and consequently underestimate long movements inside forest. A trapping grid inside forest with traps at 20 meters of each other would have been more adapted to evaluate dispersal movement inside this habitat. Don't you think this could be a bias in estimating the ratio of inside-forest versus road-crossing movements on which is based the barrier effect ?

Otherway, it would have been perhaps usefull to discuss more about the kinds of movements that this sampling design permits to reveal. Small mammals exhibit both foraging and dispersal movements. Foraging movements may be of different length according to food availability but occur mainly inside the optimal habitat of the species whereas dispersal movements are often induced by intra specific interaction and correspond to settlement of individuals in new areas. In this latter case, individuals are more prone to cross unoptimal habitats such as meadows, crops or bare ground where predation risk is greater and which may be perceived as barrier as well as roads. So your data would certainly be confortd by comparing movements ratios at different types of forest edges. For example, a wide grassland habitats may be of higher predation risk than a narrow road and so a stronger barrier to dispersal. This would have help to evaluate relative barrier effect of roads.

The second paper focused on the barrier effect of highways of more than 40 m wide for the two rodent species and tested homing ability of displaced individuals from one to the

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other side of the road. The author found that middle width highways did not prevent movements of the two species, although bank voles crossed only after translocation. On the other hand, very large highways prevent crossing of the two species.

Very low numbers of voles and mice crossed the highways and the wide county road but one more time I have little doubt about sampling design efficiency to evaluate the barrier effect. As road width ranged from 19 to 50 meters why don't you set trap lines inside forest at these same distances or on different grid sizes simulating road width. I think this sampling design would have been more efficient to compare trapping rates in the two directions (inside forest and across the road). As rodents had high probability to be intercepted by traps set 5 meters apart of each other, you can't reject the risk of underestimating long movements inside forest. Inversely, at the forest edge where traps were settled, individuals may display lot of parallel movements to the road leading to an overestimate of movements length in this area.

Experiments on transferred individuals demonstrated clearly differences in crossing ability of the two species and reinforce the fact that, in case of high motivation level to cross, roads may not act as barrier. This opens to a new perception of the barrier effect. The new question arising would be : when an individual is highly motivated to change its home range, do roads act as real barriers to movement ? If not, roads should be considered only as unfavorable habitats with high predation and killing risk but perhaps no more than other non forest habitats. In any case, as non optimal habitats they increase the fragmentation of forest.

The third paper aims to demonstrate that the barrier effect of highways that prevented interchange between individuals of the two rodents species could induce genetic subdivisions of their populations. The genetic structure is based on DNA microsatellites analysis. The author found high genetic diversity in both populations of bank vole and Yellow-necked mouse. Allele frequencies in both species indicated that sampled subpopulations did not belong to a single random breeding population but that differentiation were more marked along the eastern-western gradient than along the northern-southern one delimited by the highway. Populations of both species showed genetic structure in accordance with a typical isolation by distance model. Finally, no significant difference were found for populations living at opposite sides of the highway. In the discussion the author formulates interesting hypothesis based on life traits of species, age of highway and underpass structures that could explain these results. This study is well done using genetic as well statistics robust and adapted methods and does not suffer of any weakness. Maybe the absence of genetic structure associated to the presence of the highway would indicate that capture-recapture works from previous paper were not sufficiently efficient to evaluate crossing rate movements.

Although, other sampling designs and comparative analysis of the barrier effect of different kinds of habitats would have brought interesting complementary information on the real negative effects of roads, this thesis, as a whole, furnishes interesting and new complementary results. This work contributes to better understand consequences of habitat fragmentation induced by road development. The thesis is carefully written and two of the three papers have already been evaluated and accepted by the scientific community. I strongly feel this work is good and discussed with high scientific rigour. I suggest it to defense by Adriana RICO.

Rennes, 2007-01-25  
Alain BUTET



A review of the PhD thesis by Adriana Rico „Effect of roads on small mammal populations“, University of South Bohemia, Faculty of Biological Sciences, České Budějovice (2006).


The evaluated thesis consists of a brief introduction and manuscripts of three research papers that were submitted or accepted for press in international journals. All the included papers are aimed to the investigation of effects of roads as barriers of movement in small mammals. In this respect, the thesis is thematically consistent and the context of individual topics is clear.

The introductory part of the thesis includes the abstract (1.5 p.), Introduction including a characteristics of the species studied (4.5 p.), the scopes of the thesis (0.5 p.), conclusions (< 1 p.), and references (5.5 p.). It seems that the content of this part is not well-balanced, and I would prefer to get more detailed information about the actual results of the thesis and their interpretation. Almost no synthetic comprehensive summary of the research performed is presented.

The manuscripts included in the thesis have been sent to distinguished journals, they are based on rich materials, and their results are remarkable in many points. My impression is that the second paper (*Acta Theriologica*) is the best one, whereas the third manuscript (*Molecular Ecology*) seems to me rather premature for publishing. Individual remarks and comments to the introductory part and the manuscripts are listed below.

Generally, I can evaluate the thesis as a valuable and original document for the PhD defence and I can recommend it to be accepted for the doctoral procedure.

Brno, February 4, 2007

  
prof. Jan Zima

#### Individual comments and remarks:

**Abstract:** It is too long for a standard abstract, and it reports data that are usually not included in abstracts. On the other hand, information on own original results might be more comprehensive.

#### Introduction

The concept of „internal fragmentation of forests“ is derived mainly from studies conducted in virgin tropical rainforests, and it is questionable if it may be directly implied to the Central European landscape.

The arrangement of references in the text is rather chaotic (references are not arranged alphabetically or strictly chronologically in the parenthesis).

The edge effect need not cause lowering of the biodiversity level; on the contrary its effect is often quite opposite.

The spelling of certain geographic or personal names is erroneous (e.g. Zeida).

It is not quite correct to characterize the bank vole as „a generalist vegetarian“. The widening of the food spectrum in the species is primarily caused by a high intake of animal food in spring.

The breeding of yellow-necked mouse is in our country terminated usually several weeks earlier than the breeding of bank vole, in late summer or early autumn. An opposite situation is suggested on p. 9 and 10.

#### Conclusions

This section is in fact a shortened abstract. The idea that mice can cross highways during traffic mess sounds funny.

#### References

The paper by Gerlach a Musolf (2000) is missing, in spite of being one of the most important references in respect of the aims of the thesis.

#### Paper 1

Rico, Kindlmann, Sedláček

Barrier effects of roads on movement of small mammals.

Folia Zool. in press

#### Abstract, p. 18

The common shrew should not be listed among forest rodents.

#### Material and Methods

The differentiation of roads according to their widths is not unequivocal. The values of the maximum road width (including verges) are overlapping.

What is the source of data about traffic density? Are these data derived directly from the sections of roads under study? Was the density measured also during night hours?

Fig. 2. Colour differentiation of columns in the figure is chaotic and it does not correspond with captions.

#### Results

p. 23-25

The sex ratio in the samples obtained does not correspond to usual situation in populations of the species, when adult males prevail in spring and in the beginning of summer, whereas slight prevalence of females or the balanced sex ratio is found in autumn. What is the explanation for this deviation? What was the age structure and sex ratio in the samples of individuals that had crossed the road (Table 3)?

p. 25

Migrations of individuals up to several km were reported in previous published studies. It is questionable if a movement involving several tens or hundreds of meters can be designated as long.

s. 26

The differences that were not proved as significant should not be considered as any differences (e.g. no difference was evidenced in migrations of bank voles across narrow and wide roads).

The effect of tree canopy is not reported in the Results section.

#### Discussion

p. 27

The first chapter seems to be redundant, it repeats known facts or information presented earlier in the manuscript.

I would not consider the recorded densities as low because other reported densities are generally even lower in Central European landscape.

The discussion appears rather superficial, and it is dissected into many particular problems that are often examined speculatively.

#### Literature

The references are not arranged strictly alphabetically.

#### Paper 2

Rico, Kindlmann, Sedláček

Road crossing in bank voles and yellow-necked mice.

Acta Theriologica (in press)

p. 34

I am not sure if the area under study can be characterized as „deciduous pine dominated forest“.

p. 35

Data of trapping trips should be mentioned.

p. 35-36

How the adult and subadult animals were differentiated?

p. 41

Providing that the two parallel trapping lines were separated by only 5 m, it is problematic to derive conclusions about differences in home ranges or migration tendencies of animal captured in both respective lines.

p. 42

The traffic density of 2000 cars per hour is obviously an average value of the whole day period (24 hours). The night density was apparently considerably lower.

It is not relevant in the studied species to relate the barrier effect of the highway to the inbreeding increase and the risk of local extinction.

p. 43

The breeding season in the yellow-necked mouse is usually terminated considerably earlier than at the end of October.

#### Práce 3

Rico, Kindlmann, Sedláček

Can the barrier effect of highways cause genetic subdivision in small mammals?

Molecular Ecology (submitted)

#### Introduction

The studies reporting the barrier effects of roads were often performed in region with quite different ecological and geographical properties compared to the area under study (Dale et al. 1994, Laurence 1997, Goosem 2001, Burnett 1992, Lovejoy et al. 1982). The only relevant study for the present paper seems to be Gerlach and Musolf (2000).

p. 49

„Rico et al. (2007) observed that populations were **not able to cross** a highway“. This is not true, because three cases of crossing the highways were reported in the previous paper.

s. 51

Geographic position of the sites studied along the highway is incorrect in Fig. 1 and does not correspond to the description presented in the text. The sites HW1 a HW3 are obviously confused. This is almost impossible to understand properly the key part of the Results section on p. 58-60 and data in Table 3 without realizing this mistake.

The abbreviations used are not properly chosen. E.g., the symbol HW designates either the Hardy-Weinberg equilibrium or the highway.

p. 55

No variation was found in a microsatellite locus in yellow-necked mouse, and the data from this locus did not contribute to evaluation at all. It would be more correct to report four loci employed in determination of the genetic structure. Was the respective locus reported monomorphic also in other published studies?

p. 58-60

Data in Table 4 are not clearly interpreted. Their understanding is further complicated also by the confusion of the sites HW1 a HW3 in Fig. 1, as noted above.

The geographic distribution of the sites studied is not suitable for a phylogeographic analysis in the area under study. This is apparently a consequence of the design of selection of studied sites that was originally not focused on isolation by distance or phylogeographic pattern investigations. What is the significance of differentiation of clustered groups in the sample studied?

p. 60, Fig. 2

The divergence between populations on the north side of the highway was found more distinct compared to the opposite south side. This is further explained by heterogenous landscape on the north side. Are there any exact data evidencing this difference in the landscape heterogeneity on the both sides of the highway?

p. 61

How the heterozygote deficiency or inbreeding extent could be influenced by the presence of null alleles?

p. 63-64

The higher level of differentiation between populations of the yellow-necked mouse compared to bank vole mouse is really surprising. How the species status of mice collected was checked? Was the presence of a morphologically similar species (*Apodemus sylvaticus*) definitively excluded?

General remarks to the paper 3

This study originates from a well-designed experimental investigations and it employs advanced techniques of analysis of highly polymorphic molecular markers. It is quite surprising that this study resulted in evidence for no detectable effect of the highway on the gene flow between populations of small mammals on its opposite sites. This conclusion is even not congruent with the findings of previous study made in the same sites that demonstrated rather limited crossing of the highway. The discrepancy between expected and revealed data has not been satisfactorily explained in the manuscript. I do not think that the „escape“ to discussion of isolation by distance was reasonable in this respect. On the other hand, the finding that the highway with very high traffic density actually does not represent any barrier for dispersal of small mammals is quite important. If the authors believe in their data, they should defend this actual message of the paper, and not to turn towards rather speculative discussion about isolation by distance effects. Perhaps the conclusion that barrier effects of highways are only mythical should be further corroborated in following sampling and analyses. In this respect it seems me that publication of the paper may be premature.



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A critical review of a doctoral dissertation

### **Effects of roads on small mammal populations**

submitted by Adriana Rico Cernohorska  
supervised by prof. RNDr. Pavel Kindlmann, DrSc.

Fragmentation and habitat loss are processes occurring almost everywhere. Ultimately, they increase the risk of extinction for many species through a reduced population size and genetic variation. Internal fragmentation has been coined to refer to natural habitats subdivided by linear barriers, such roads, railways or power lines, restricting further animal movement within habitats. To study effectively these processes, it is advantageous to apply simultaneously two approaches, demographic, to focus on movements of animals across a barrier, and genetic, to get insights into genetic structuring resulting from disturbed demography in a longer run. I am very delighted to see that both the approaches were employed in a doctoral dissertation submitted by Adriana Rico, studying the barrier effects of roads on small forest mammals. This combination is important as the movements alone do not translate directly to a gene flow and gene flow tells us nothing about the movements.

The dissertation is based on 3 papers in international journals (*Folia Zoologica*, *Acta Theriologica* and *Molecular Ecology*), with some introductory stuff of about 6 pages. Two papers are in press, the third one was submitted and is being reviewed. While the first two are concentrated on demographic processes, concentrating on studying frequency of movements across roads and highway, the last examines the barrier effects on genetic composition of populations on both sides of the road.

In Introduction, a brief review of negative effects of roads on animal movements is given, along with short overviews of two main mammals studied, the bank vole and yellow-necked mouse, ending with a section summarising the major results. Whereas county roads act as a penetrable barrier for small rodents, being more penetrable for mice than voles, the highways seem to prevent movements completely in both species. This is bad news. However, genetic investigation was unable to demonstrate that the populations are subdivided, suggesting that the rodents can find some way of crossing the highway. This is good news.

I appreciate much that the thesis is one coherent story, documenting a well-considered and ingenious research project. It combines demographic methods with modern molecular techniques to give insights into both ecological and evolutionary processes. All findings are carefully tested statistically and interpreted in a balanced way. The doctoral thesis is written in good and readable English. Thus far, I was mostly positive. The second part of my review is concerned with some criticism and questions. However, I stress that my critical comments should better be considered as





suggestions for further research rather than an attempt to devalue the significance of the presented work. As my expertise is not much on the side of genetic analyses, I will stick to papers I and II.

In both of them, I did not understand, and ask question about that point (1), why population size was not computed using probabilistic models such as those implemented in the program Capture for closed populations, especially if capture-recapture data were available. Instead, simple population index was provided which is known to suffer from many deficiencies. The major one is that it assumes the same and constant probability of capture across all sites. This is hardly the case as capture probabilities are known to vary in less predictable manner, depending on many factors, such as weather or demographic structure. Producing first the estimate of capture probability, the probabilistic models are able to correct the counts of the animals captured for unequal catchabilities to properly reflect abundance in a way that permits comparison of abundance over time and space. Moreover, any population proportion, such sex ratio or the proportion of animals crossing the roads, calculated directly from sample counts is very likely to be biased (3). Therefore, the proportion of individuals crossing the road is most likely to be higher than presented because capture probability is frequently much lower than 1. More importantly, new modelling approaches have been developed specifically for landscape studies in small mammals, such as multistate models, that can model directly probabilities of movement between two patches. Consequently, I strongly recommend that you should taken pains and put probabilistic modelling on your own list of mastered techniques to increase further the rigor of your scientific work in future research.

I also have few comments to statistical analysis. When dealing with population data, it is almost a rule that the data are log-transformed prior to analysis (3). This is because the variance is always related to the mean as a consequence of a multiplicative nature of population processes. In other words, log-transformation is used to stabilise the variance in data, the important assumption of most statistical analyses, ANOVA in particular. Furthermore, ANOVA and contingency tables, as being used heavily in both papers to analyse variation in indices and animals counts, are nowadays usually replaced by more effective statistical tool based on likelihood, such GLM or GLMM, allowing tailoring more appropriate models to closely fit the sampling design of data (4). These models are capable of analysing the data set as a whole, not in a piecewise manner as practiced in both papers. In addition, when testing demographic structure, it does not matter whether population sex ratio deviate from parity (5). What is of great importance to us, however, is to be sure that the populations from different sites do share the same structure. Population sex ratio varies seasonally in voles, so there is no reason to expect the equal numbers of sexes.

Curiously enough, it was a little bit surprise to me to see that the main conclusion reiterated at the end of the paper I is that the roads have a species filtering effect but no age or sex filtering effect (6). This conclusion is remarkable because no data on age or sex were analysed in that paper. Perhaps, this criticism is more pertinent to the quality of the review process than to authors themselves.

In conclusion, I have two questions. The first is related to the road width. Did you notice any differences in road-kills on highways compared to roads of a narrower width (7)? The second, there was no reference to large fluctuations in vole population numbers in paper III when discussing the obvious discrepancy between the rates of ecological processes and their genetic consequences and when explaining the smaller degree of genetic structuring in voles compared to mice (8). Could it be that these more or less regular outbreaks could contribute to more homogenous populations in voles?

### **Conclusion**

Based on reading critically the dissertation and given the criteria approved for Ph.D. dissertations in the Czech Republic, I came to the firm conclusion that the honoured candidate, Adriana Rico Cernohorska, has managed to demonstrate in her dissertation the scientific rigor, mastery of the topic and skill to apply scientific research methods. Consequently, I recommend the dissertation for a public defence.



Emil Tkadlec  
Professor of Ecology  
PřF UP Olomouc  
January 31, 2007, Olomouc