tussen Duin & Dijk

Connection and defragmentation

Nature in Noord-Holland. Volume 17 3 ● 2018

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Ecoducts and genetic exchange among ground beetles

To investigate whether ecological connections actually lead to genetic exchange, in 2017, University of Amsterdam student Jesper Brugman performed a population-genetic study of ground beetles in the Zuid-Kennemerland dunes.

● Amara convexior. *Photo: Theodoor Heijerman.*

●*Research area with sampling locations.*

The main purpose of establishing an ecological connection is re-establishing (genetic) exchange between populations that due to habitat fragmentation have come to live in isolated areas. Despite often intensive monitoring of the use of ecoducts by individual animals, the effectiveness of the ecoducts with regard to their main objective has hardly been studied, if at all. The construction of three ecoducts in the Zuid-Kennemerland dune system combined with the monitoring of ground beetles (*Carabidae*) around these ecoducts by Boeken (2015, 2016) have created a great opportunity for research based on the following questions:

-Do the motorways and railway lines that these ecoducts have been/are being built across form a barrier for genetic exchange between populations of ground beetles on either side?

-Are there any indications that the ecoduct that has been constructed has improved genetic exchange among ground beetles?

Research design

In 2017, a student at the University of Amsterdam performed research aimed at answering the above questions. Ground beetles are a suitable animal group for this study, because (a) they have a short generation time, and (b) there are winged, mobile species as well as wingless, less mobile species. Feature (a) ensures that the effects of limited

● *Calathus fuscipes. Photo: Theodoor Heijerman.*

genetic exchange or a reversion will be measurable sooner, while feature (b) makes it possible to compare the effects of barriers for mobile and less mobile species, while the assumption is made that mobile species will be less hindered by the barriers.

Sampled areas

Monitoring of the ground beetles takes place by means of trapping cups ('pitfall traps'), which are placed at a site by digging them into the soil at 5 m intervals. Typically, the cups will contain formalin, which kills the trapped animals quickly and preserves them for later identification. Unfortunately, formalin does have a negative effect on the quality of the DNA. Other options are glycol or salt water, but their effects on DNA quality are unclear. The decision was made to use 'dry' pitfall traps without any liquid, in which the animals stay alive (for longer) and the DNA is preserved better (cf. Keller & Largiadèr, 2003). The dry cups do have to be emptied more often, however.

The research area (map) was divided into four main areas, intersected by the supposed barriers Zandvoortselaan (Amsterdamse Waterleidingduinen (AWD) and Koningshof), the Overveen-Zandvoort railway line (Koningshof and Kraansvlak) and the Zeeweg (Kraansvlak and Kennemerduinen). At the time of this study the Zandpoort ecoduct passing over the Zandvoortselaan had been in use for four years, the Zeepoort ecoduct passing over the Zeeweg had just been finished and preparations for the Duinpoort ecoduct passing over the railway line were underway.

Sampling genetic diversity and how it is related to genetic exchange

For the purpose of sampling the beetle populations, each main area was divided into two or three sub-areas. In each of these sub-areas, 20 pitfall traps were dug into the soil that were used for sampling from mid-May through to mid-July. The purpose of the sub-areas is being able to compare the differences in genetic diversity between sub-areas within a main area to the differences between sub-areas separated from each other by a barrier. This kind of set-up is necessary to make a statistically reliable judgement on the effects the barrier has on genetic exchange. The main assumption is that populations in two sub-areas that regularly exchange individuals (and hence genes) are genetically much more similar than populations in two sub-areas in which the genetic exchange is hampered by the presence of a barrier.
The genetic diversity of the ground beetles was determined using so-called AFLP markers. These are random bits of DNA that are used to construct a 'DNA fingerprint' for each individual.
Another advantage of sampling several sub-areas per main area is that they provide information about the presence of beetle species outside the areas already sampled by Boeken (2015, 2016) on or in the immediate vicinity of the ecoducts. This information can be used to better assess whether ground beetle species that were initially only found on one side and later also caught on the other side of an ecoduct actually must have used the ecoduct to get there. After all, they may have also come from other parts, further removed from ▶

	AWD			Koningshof		Kraansvlak			Kennemerduinen		
Scientific name 1	2	5	3	4	6	7	8	9	10	total	
Amara aenea	3						1	1			5
A. bifrons	2				2						4
A. communis				1							1
*A. convexior**	16		9	1	5	18	11	13	6	13	92
A. curta	3	12	8	8	3			2	8		44
A. lucida		1	5	2					3		11
A. lunicollis	2		1								3
A. ovata									1		1
A. spreta						1			1		2
Calathus ambiguus		1	2						3		6
C. cinctus		2							1		3
C. erratus				1							1
*C. fuscipes**	1	3	2		19	13	4	2	3	1	48
C. melanocephalus			2						1		3
Carabus nemoralis						1	1		1		3
Cicindela hybrida		2									2
Harpalus anxius	3	4	1	2		1			1		12
H. pumilus	2	2		2	1	2	4	1	4		18
H. servus		11	14	5					1		31
H. tardus	5	1	1	20	10	1	3	3	6	3	53
H. xanthopus					1						1
Masoreus wetterhallii	3	1		4	5			1	1		15
Panagaeus bipustulatus	1					2				1	4
Poecilus versicolor						6				5	11
Syntomus foveatus			5								5

Table 1. Overview of the ground beetles that were caught per area and sub-area. See map on page 34 for the sub-area numbers. * = genetically analysed.

the ecoduct, of this area that first appeared empty.

Results

Though a reasonable spread of (25) ground beetle species was found (table 1), the numbers for the individuals caught in each sub-area were very disappointing, despite the large amount of pitfall traps. A fair sampling of the genetic diversity of a sub-area requires a DNA fingerprint constructed from a minimum of 10 (preferably 20-30) individuals. Such numbers were only achieved locally for *Amara convexior* and *Calathus fuscipes*, which are two fairly common ground beetles in the research area. Reasonable numbers of *Harpalus tardus* and *Amara curta* were caught as well, but only in the main areas of Koningshof, AWD and the Kennemerduinen respectively. This meant that for these species, no meaningful statistical comparison could be made, neither between sub-areas within a main area, nor between main areas.

Only *A. convexior* and *C. fuscipes* have been genetically analysed; the first species for sub-areas 1 (AWD), 6, 7, 8 (Kraansvlak) and 9 and 10 (Kennemerduinen), and the latter for sub-areas 4 (Koningshof) and 6 (Kraansvlak). Through this analysis, it became possible to assess for *A. convexior* whether the Zeeweg, the railway line and/or the Zandvoortselaan had been a barrier. With the available samples for *C. fuscipes*, only the effect of the railway line could be assessed.
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Although the individuals within the sampled populations show considerable genetic diversity, the sub-populations are all very similar to each other. This is evident from a value for genetic differentiation, F_{ST}, which for both species is not statistically significantly different from zero (the F_{ST} for both species is 0.003). This result indicates that there is probably considerable genetic exchange between the sub-populations. There are no indications that the roads or the railway tracks constitute, or have constituted, a barrier for these two ground beetle species.

Discussion

Knowledge on further distribution

The sampling of ground beetles in a larger area allows for a better interpretation of species' movements across the Zandpoort ecoduct.
Yet even though more extensive sampling was attempted, compared to previous research, far fewer species have been found: 25 instead of the 68 that Boeken found on and around the three ecoducts in 2017. The lower species diversity is probably due to the fact that the trapping period was shorter and only one habitat type, open dune grassland, was chosen. All species were also previously caught by Boeken, although not all in the same sub-area. Without further knowledge on the wider distribution of species across the whole area, the monitoring, which is concentrated in the immediate vicinity of the ecoduct, may give the impression that species cross the ecoduct and colonise the new area. Boeken (2018, this issue)

● *Sorting the ground beetles on site. Photo: Jesper Brugman.*

gives an example of *Masoreus wetterhallii* only being caught on the northern side of the Zandpoort in 2015, and then also on the ecoduct in 2016. In 2017, this species was also caught on the other side of the ecoduct, so it would appear that it had crossed the ecoduct. The present study has shown, however, that this species was also present in the AWD, to the south of the Zandpoort, in two of the three sampling sites. This means we cannot conclude that the species travelled from north to south. We can, however, assume that individuals that eventually migrate across the Zandpoort (can) effect genetic exchange between populations in the AWD and Koningshof. This makes this non-flying species a good candidate for follow-up research.

Research into genetic exchange

Despite the relatively great effort that went into sampling, it turned out to be difficult to capture enough individuals for a good genetic analysis for the entire area. For the two species that have been studied, no evidence was found that the roads and railway tracks in the dunes have negatively affected genetic exchange between sub-populations. However, these two species were not the most ideal candidates with which to answer these questions, because they are among the most common species in the area. The latter already indicates that they are successful when it comes to dispersing, so the odds that they are affected by barriers are also smaller. *Amara convexior* is a winged species, and also not picky with regard to its habitat (eurytopic), as long as it is dry. And while *Calathus fuscipes* cannot fly, it walks quickly and is also extremely eurytopic (Turin, 2000). Consequently, both species will most likely find it relatively easy to cross a road or railway tracks.

Research method

Our study shows that the sampling effort must be improved, so that sufficient samples for a good analysis may still be collected for less mobile and less common species. Better traps will have to be used as well, with perhaps a preservative inside after all. Many catches were lost this year because foxes got access to the cups and possibly also because beetles were able to fly or to walk out or eat each other. 'Dry' cups should actually be emptied even more frequently, which in 2017 did not always prove to be possible logistically. Research into the effects of different preservatives on the quality of the DNA in the captured beetles may help to further optimize the method.

Although it would not have made much of a difference for the investigated species, it would be desirable for follow-up research to have markers with a higher resolution. The 'dominant' AFLP markers that were used here may have been inexpensive, but also come with disadvantages. Other markers, such as microsatellites or single-nucleotide polymorphisms (SNPs) provide more detailed data, but have to be developed separately for each species. That was not feasible within a student-led pilot project like this.

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