The Basis of Reproductive Isolation Between Ecologically Divergent Host Plant Races

Author: Emma Kärrnäs

Supervisor: Anna Runemark

Co-advisors: Jesús Ortega and Magne Friberg

BIOK01 2018, 15 credits

2018-08-31

ABSTRACT

Insects and their host plants constitute a considerable proportion of the diversity on earth, and host shifts hence provide excellent study systems to study the process that drive diversification. In this study, I address what the mechanism to achieve assortative mating is in two host races using different host plants to increase our understanding of how reproductive isolation can arise in the face of ecological specialization. The focal species *Tephritis conura* is a peacock fly that has recently diverged into two host races through a host plant shift from *Cirsium heterophyllum* to *C. oleraceum*. I performed behavioral trials on the two host races, in sympatric and allopatric populations, to test host plant preference and the importance of both visual and olfactory cues for identifying and choosing host plant. I found that the flies preferred the scent of their host plant and spent significantly longer time in the part of a Y-maze with the scent from their host plant. Conversely, flies did not spend significantly longer time in a compartment with visual contact with their host plants. *T. conura* is thus able to identify their host plant, indicating that host plant preference acts as a pre-mating isolating barrier between the two host plant races. If there would be stronger selection for a plant preference in sympatry to avoid maladaptive hybridization we would expect the preference to be stronger in sympatry than allopatry, but I did not detect any such difference in preference strength between the sympatric and allopatric population with *C. oleraceum* as host plant with the sample size used in this study. Studies identifying the genetic basis of host plant adaptation and preference would be interesting to further understand how mate preference is coupled to performance differences on host plants during host plant driven diversification.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Reproductive isolation and speciation

The beauty and diversity of life on earth has always captured and puzzled us. When Darwin published his book *On the Origin of Species* in 1859 he revolutionized not only the field of biology as a science, but also challenged our perception of the world and its biodiversity. There is today consensus that ecological selection is the main driver of speciation (Schluter 2009). Allopatric speciation has traditionally been thought to be the most common form of speciation, but the relative importance of and the mechanisms that drive sympatric speciation is still in need of further investigation. In allopatric speciation models, reproductive isolation is a consequence of selection and/or genetic drift, while in current sympatric speciation models this is due to restriction in gene flow through disruptive selection (Diegisser et al. 2006).

Divergent ecological selection may cause populations to become distinct over time, both phenotypically and genetically. These changes might in turn lead to reproductive isolation and thus new species (Nosil 2012). How associations between traits under divergent natural selection and reproductive isolation, i. e. "magic traits", arise is a matter not well understood (Barton 2010; Nosil 2012). Evolution of traits under divergent selection may lead to non-random mating as a by-product, and therefore lead to pre-mating isolation (Nosil 2012).

Selection against interspecific mating will occur if matings between populations adapted to different niches generate offspring with lower fitness, compared to matings within the populations. If there is selection to avoid mating with individuals with different niches reproductive character displacement can arise as a consequence of this reinforcement. In this case prezygotic isolation will be stronger in regions where costly hybridization is possible, i.e. where populations are sympatric or parapatric, compared to non-hybridization regions where populations are allopatric (Servedio and Noor 2003).

1.2 Insect and host plant interactions

Insects and their host plants constitute a considerable proportion of the diversity on earth, and host shifts hence provide excellent study systems to address diversification (Bernays and Graham 1988; Diegisser et al. 2006). Speciation in sympatry due to host plant shifts have been shown in both empirical and theoretical studies, although it is hard to entirely rule out allopatric or parapatric phases during the process (Johannesen et al. 2010). The tephritid fly *Rhagoletis pomonella* is a model species for ecological speciation by such host plant shifts, where the populations have evolved to use two different hosts (Tait et al. 2016).

The majority of phytophagous insects only oviposite on a few host plants that are often closely related, belonging to the same genus or family. Potential factors that restrict the host range are natural enemies, interspecific competition, physiological constraints, host plant morphology and phenology. However, ultimately the reason of determination of a species host range is the behavior of the insect; the female's choice of host plant (Diegisser et al. 2009). It is critical for insects to being able to locate and distinguish their preferred plants for feeding, mating and ovipositioning (Tait et al. 2016). When searching for a food source, syrphid flies are attracted to yellow flowers in particular, indicating that visual stimuli is an important factor. However, the syrphid fly *Episyrphus balteatus* have been shown to rely on olfactory cues rather than visual to find the pink flowers of *Cirsium arvense* (Primante and Dötterl 2010). Flies of the family *Muscidae* are able to locate carrion flowers without visual cues, although they easier locate them with the combination of visual and olfactory cues (du Plessis et al. 2018). The two distinct host plant races of *Rhagoletis pomonella* has been shown to find their host plants by only olfactory cues (Tait et al. 2016).

Here, I take advantage of a peacock fly species, *Tephritis conura*, that has recently diverged and specialized on two host plant races to address 1) whether host plant preference is an isolating barrier in this system, 2) whether visual or olfactory preferences are important for identifying host plants and 3) whether there is evidence for reinforcement in sympatric populations.

2. MATERIALS AND METHODS

2.1 Study system

The focal species *T. conura* is a fly recently diverged into two host races through a host plant shift from *Cirsium heterophyllum* to *C. oleraceum. T. conura* is a tephritid fly able to use different species of the thistles in the genus *Cirsium* as host plants (Diegisser et al. 2006). Nine *Cirsium* species have been recorded as host plants: *C. heterophyllum, C. oleraceum, C. palustre, C. acaule, C. spinosissimum, C. erisithales, C. filipendulum, C. canum* and *C. pannonicum.* However, not all of these species have been demonstrated to have specific host races. In addition to the strains of flies mainly infesting *C. heterophyllum* and *C. oleraceum*, the population facultatively infesting *C. palustre* and *C. heterophyllum* in Scotland might also be distinct (Diegisser 2005).

The flies mate and oviposite on their host plants in late spring, when the flowers have buds (Diegisser et al. 2006). The buds need to be at a specific stage of development for being suitable for ovipositioning; the cavity in the buds need to reach a certain size. The larvae feed on the host plant and pupate and emerge as adults. The two host plants differ in both morphology and phenology. In *C. heterophyllum* flies emerge in July and in *C. oleraceum* the flies emerge mainly in August. Next spring the adults mate and lay eggs on their host and die shortly after (Diegisser 2005). Flies emerging from *C. heterophyllum* and and *C. oleraceum* differ genetically, suggesting there are different host races. Analysis of mtDNA sequences suggest the diversification has occurred recently (Diegisser et al. 2006). Flies emerging from *C. oleraceum* have longer ovipositors, compared to flies emerging from *C. heterophyllum* (Diegisser et al. 2007). Interestingly, the survival rate of the offspring of *C. heterophyllum* flies on *C. oleraceum* was significally lower compared to offspring of *C. oleraceum* flies on *C. oleraceum* (Diegisser et al. 2008).

C. oleraceum is found in large parts of central Europe while *C. heterophyllum* in general has a more northern distribution, with their geographical distribution overlapping in certain areas. These contact areas are sympatric zones of *T. conura* populations, while the geographically separated areas are allopatric zones (Diegisser 2005). Here, the geographical distribution of the different populations was used to test whether sympatry affects host plant choice.

2.2 Sampling design

All *Tephritis conura* used during trials were caught in the wild between 5th and 13th of June 2018. Allopatric flies with *C. heterophyllum* as a host plant were sampled in Northern Sweden, sympatric populations in Southern Sweden and allopatric populations with *C. oleraceum* as a host plant in Germany (Figure 1). The flies were kept in netbags in a climate chamber set at 8 degrees with light between 09-17. They were given water and honey *ad libito* and taken out every day to room temperature for feeding. Before trials, they were taken out for acclimatization. Each fly was used solely for one trial, either olfactory or visual. They were kept at a maximum of 5 days in the climate chamber before trials. The thistles used in the visual and olfactory trials were collected from several locations in Sweden during June.

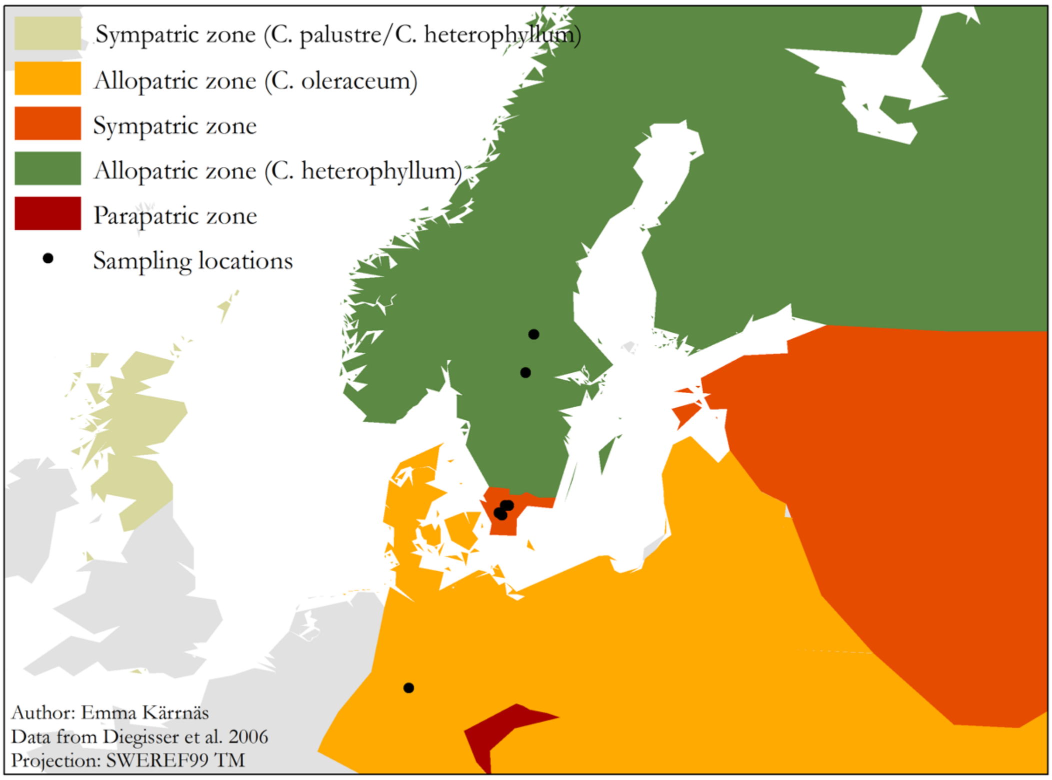

***Figure 1.* Host plant distribution and sampling locations of the *T. conura* used during visual and olfactory trials. The sympatric (orange) and parapatric (dark red) zones not specified are areas where both *C. heterophyllum* and *C. oleraceum* host races occur.**

2.3 Trials

All individual trials lasted a total of 300 seconds. The trials were conducted outside at 25°C in the shade during the first day, from 10-15. All other trials were done inside at room temperature, between 08-17. Trial order was randomized in terms of treatment, sex, host plant race, sampling site and the sides the thistles were on. Controls were blank; no plant at all were used during the visual trials and a ball of aluminum wrapped in plastic was used during the olfactory trials.

2.3.1 Setup for visual trials

We used a closed T-maze made of UV-transparent perspex, consisting of three compartments; mid, left and right (Figure 2). One species of *Cirsium* (*heterophyllum* or *oleraceum*) was placed at either side of the tube, their buds visible but physically separated from the interior of the tube. The mid compartment had a hole where a smaller tube was inserted, containing the fly. Time was measured from when the flies started to move around in the initial tube. Time spent in each compartment was measured; the mid compartment was decided to be neutral ground.

Figure 2. **A closed T-maze made of UV-transparent perplex was used for the visual trials. It was made up of three compartments; mid, left and right. In this case, *C. oleraceum* is seen by the flies in the left compartment and *C. heterophyllum* by the flies in the right. The mid compartment, including the initial tube connected to it, was a neutral zone.**

2.3.2 Setup for olfactory trials

A set of two identical open Y-shaped tubes made of transparent glass were used (Figure 3). The tubes were in one piece but divided into three zones – neutral, left wing and right wing. A piece of white fabric was placed at the end of each tube (left/right wing), respectively. A mix of buds, leaves and stems of around 2,2-2,3 g of either *C. heterophyllum* or *C. oleraceum* were placed in plastic wrapping and then placed upon the fabric, thus allowing air to pass through but limiting visual stimuli. A battery-driven air pump was connected to the entrance of the tube and put at an airflow rate of 200 ml/min (Svensson et al. 2010). The fly was placed at the entrance and time was measured from when the fly moved out of the initial part. Time spent in each zone was measured. After being used for one trial, the glass tubes were left at 200°C in an oven for 20 minutes, to eliminate any possible odors.

Figure 3. **An open Y-shaped glass tube was used for the olfactory trials. The tube was divided into three zones: neutral, left wing and right wing. A piece of fabric was placed over the opening of the left and right wing, respectively. A sample of each thistle species wrapped in plastic film was placed over each piece of fabric. An air pump was connected to the bottom of the tube to create an airflow.**

2.4 Statistical analysis

All statistical tests and graphs were done in R, version 3.5.0 (2018-04-23). To test if the flies spent significantly more time with their own host plant, t-tests were performed for both the visual and olfactory trials. Moreover, to test if they visited their host plant more frequently and if it was chosen as a first choice, more often than expected by chance, a t-test and an exact binominal test was performed for both series of trials. To address what factors best explained how long time the flies spent at the different plants I used a model selection framework. I used linear models and tested all combinations of the predictor variables sex, host plant race and distribution (allopatric or sympatric). The time spent at the correct host plant was used as response variable. To evaluate what model that best explained the data I used Akaike's Information Criterion, AIC (Akaike 1973). A lower AIC implies a better model fit, with a difference of 2 being considered significant and simple models including fewer predictor variables considered more likely than models including more factors (Burnham & Andersson 2002). I performed these analyses separately for visual and olfactory trials. R-packages used for the tests include nlme (Linear and Nonlinear Mixed Effects Models, version 3.1-137) and lme4 (Linear Mixed-Effects Models using 'Eigen' and S4, version 1.1-18-1). For graphs the package ggplot2 (Create Elegant Data Visualisations Using the Grammar of Graphics, version 3.0.0) was used.

3. RESULTS

3.1 Visual trials

A total number of 56 flies were studied using the visual setup. Out of these 42 were trials and 14 were controls, see Table 1. For the visual trials, I did not detect any significant difference between the amount of time spent at the host plant species and the other species (t-test; t = -0.12677, df = 81.867, p = 0.8994). Furthermore, neither host plant race, sex, nor geographic distribution (allopatric or sympatric) significantly affected the choice of host plant (see Appendix 1).

Table 1. **A summary of the visual tests performed. In total 42 trials and 14 controls were completed.**

Host plant race	Distribution	Sex	No. of trials	No. of controls
C. oleraceum	sympatric	male	12	3
C. oleraceum	sympatric	female	8	3
C. oleraceum	allopatric	male	6	2
C. oleraceum	allopatric	female	5	2
C. heterophyllum	allopatric	male	6	2
C. heterophyllum	allopatric	female	5	2

The host plant was not visited a higher number of times (t-test; t = -0.47558, df = 80.981, p=0.6357) nor was it more often visited first (exact binominal test; n=37, number of successes=15, p=0.324) compared to the other *Cirsium* plant. There was no difference in time spent at the host plant during control and trial (t-test; t= -1.3371, df = 23.941, p=0.1938) as expected if the flies would be attracted to the visual appearance of their host plant, see Figure 4.

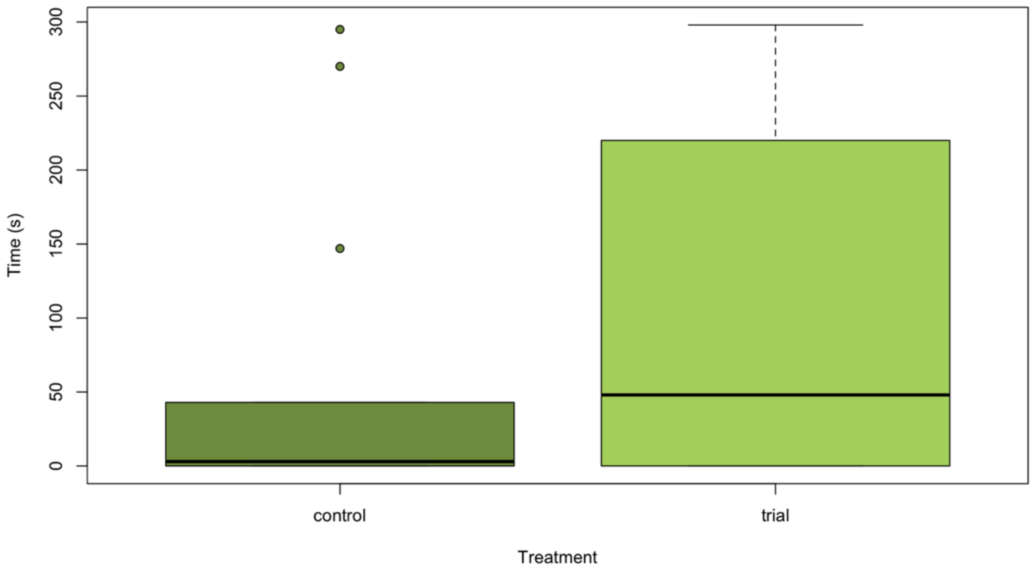

Figure 4. **Time spent at the host plant during visual trials (n=42) compared to time spent at the host plant during control (n=14). Error bars show ± 95% confidence intervals.**

3.2 Olfactory trials

A total number of 68 flies were studied using the olfactory setup, of which 56 were trials and 12 were controls (Table 2).

Table 2. **A summary of the olfactory experiments: in total 56 trials and 12 controls were performed.**

Host plant race	Distribution	Sex	No. of trials	No. of controls
C. oleraceum	sympatric	male	13	3
C. oleraceum	sympatric	female	9	2
C. oleraceum	allopatric	male	9	2
C. oleraceum	allopatric	female	5	2
C. heterophyllum	allopatric	male	10	1
C. heterophyllum	allopatric	female	10	2

Overall, we found that the flies spent more time at their host plant compared to the alternate plant during olfactory trials (t-test; t = 2.378, df = 107.76, p = 0.01917), see Figure 5. Moreover, a linear model revealed a significant difference in time spent at the two species of thistle, with both host plant races spending more time at their respective native host plants (F= 5.795, df=1,54, p= 0.01952). However, a difference between sexes could not be ruled out since the AIC value differed with less than 2 between a model including only host plant race and a model including both host plant race and sex (F= 3.163, df=2,53, p= 0.05039), see Table 3. However, while a significant difference in time spent at the host plant between *C. heterophyllum* and *C. oleraceum* flies could be observed using a t-test (t = 2.5788, df = 47.632, p = 0.01306), no such difference could be seen between sexes (t-test: t = 0.97767, df = 50.289, p = 0.3329), see Figure 6. Moreover, generally simple models are preferred over complex ones, especially when data sets are small (Burnham & Andersson 2002) and I therefore consider the model including only host plant race to be the best model.

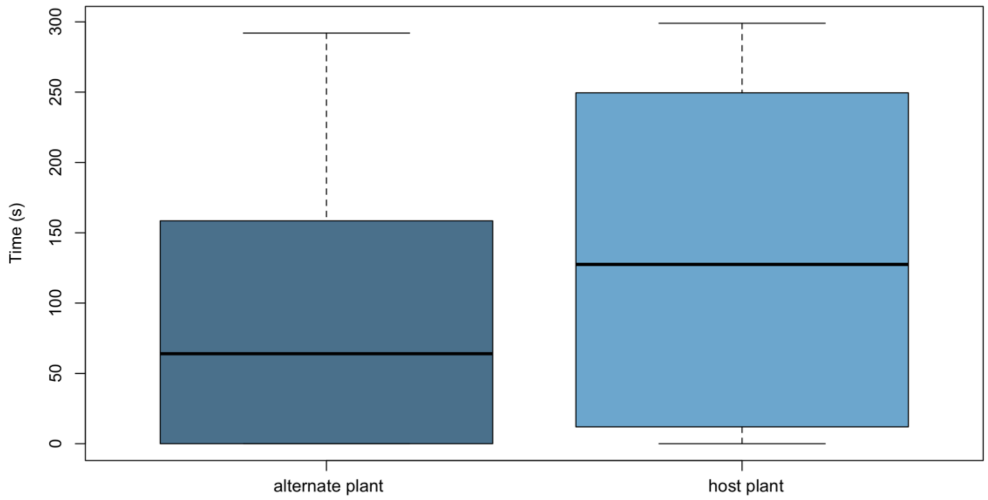

Figure 5. The time each fly (n=56) spent at their host plant and at the alternate plant, respectively, during trials. The mean time spent at the host plant was 137.4 s and the mean time spent at the alternate plant was 89.7 s. Error bars show ± 95% confidence intervals.

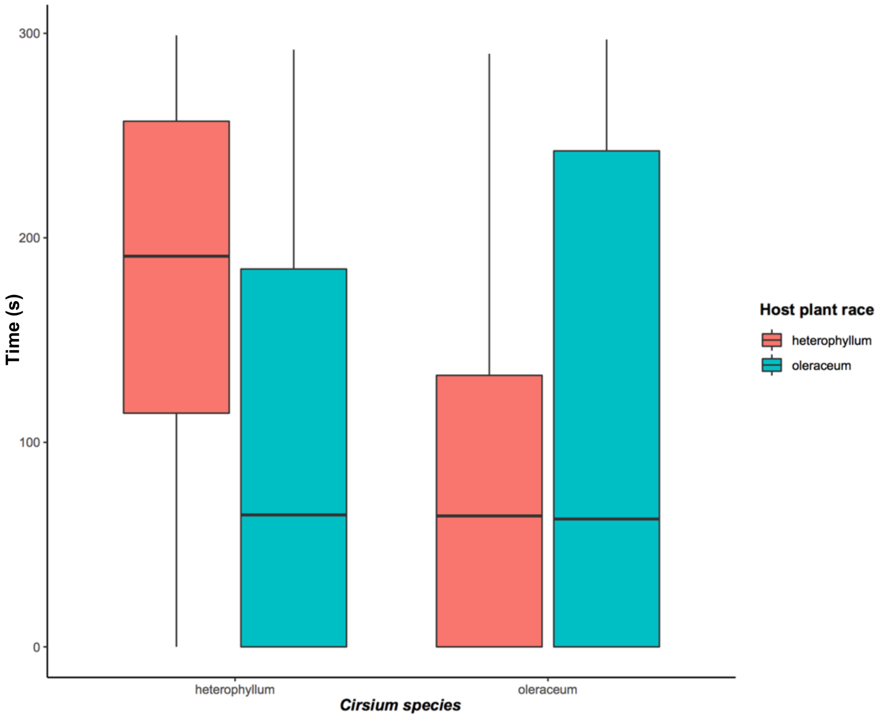

Figure 6. The time spent on the host plant by each host plant race, compared to the time spent on the other *Cirsium* species. Error bars show ± 95% confidence intervals.

Table 3. The combination of variables tested during olfactory trials, using a linear model. The best model included only host plant race. However, an additional role for a difference between sexes could not be ruled out since the AIC value differed by less than 2 between the best model and a model that included both host plant race and sex (the best models in bold text).

Variables in model	F	df	p	AIC
Host plant race	**5.795**	**1, 54**	**0.01952**	**688.0726**
Host plant race + Sex	**3.163**	**2, 53**	**0.05039**	**689.4673**
Host plant race * Distribution	2.863	2, 53	0.06594	690.0358
Host plant race + Distribution	2.863	2, 53	0.06594	690.0358
Host plant race * Sex	2.216	3, 52	0.09724	691.0440
Host plant race + Distribution + Sex	2.078	3, 52	0.1144	691.4415
Distribution	1.553	1, 54	0.2181	692.1942
Sex	0.9495	1, 54	0.3342	692.8054
Distribution + Sex	1.216	2, 53	0.3045	693.2688
Host plant race * Distribution * Sex	1.352	5, 50	0.2584	694.6821
Distribution * Sex	0.8126	3, 52	0.4927	695.2160

The host plant was not visited a higher number of times (t-test; t = 0.38873, df = 108.72, p-value = 0.6982) nor was it more often visited first (exact binominal test; n=48, number of successes=29, p=0.1934) compared to the alternate plant. There was no significant difference in time spent at the host plant during control and trial (t-test; t = 1.1077, df = 16.793, p= 0.2836), suggesting that the host plant was preferred both over no smell and the smell of the other *Cirsium* species.

4. DISCUSSION

I found evidence that olfactory cues are used to find the host plant by *T. conura* (see Figure 5). On the contrary, I found no evidence that visual cues are of any importance for identifying the host plant. Interestingly, other flies often use both visual and olfactory cues to locate flowers, although there are a few empirical studies on the topic (Primante and Dötterl 2010; du Plessis et al. 2018). Interestingly, in line with the results of this study, another tephritid fly with two distinct host plant races (*Rhagoletis pomonella*) has been shown to have an olfactory preference for their host plant. However, the importance of visual cues where not tested in this study (Tait et al. 2016).

Nevertheless, scent preference appears to be an important trait for tephritid flies. In *R. pomonella*, the different host plant races differ not only genetically but also in their olfactory preferences of host plant, which can by linked to compound olfactory sensory neurons on their antennae. These bind to specific odorants in the environments and send information to the brain, which ultimately leads a behavioral response (Tait et al. 2016). In this way, scent preference may be coupled with ecological adaptations to the host plant. For further studies it would be very interesting to test the chemical compounds associated with the host plant and compare to receptors on the antennae of *T. conura*, as done in similar studies (Tait et al. 2016; Primante and Dötterl 2010; du Plessis et al. 2018). This will pin down what receptors are involved in the differential preferences, and enable studies of the differences in the genes coding for these receptors between the host races and studying the genetic signatures of selection acting on these genes.

Interestingly, I could not detect any significant difference between populations living in sympatry and allopatry with respect to the importance of olfactory cues, and hence no indications of reinforcement were found. This raises the question if hybridization occurs in sympatric zones, as it does in some parapatric contact areas in mountains on the European continent. However, the two host plant races have different hosts, and adaptions due to this e.g. female ovipositor length appear to be longer in *oleraceum* flies (Diegisser et al. 2006; 2007) and hybridization should thus be selected against. The sympatric *heterophyllum* population was not tested because we could not obtain any individuals in field due to the abnormal weather conditions that set on a very early spring. Moreover, the low sample size in the current study may not enable us to detect smaller effects, and a more extensive study with much higher sample size would be needed to conclude whether there is any evidence for reinforcement in the *T. conura* study system. Other interesting topics to study would be both phenotypic correlations between scent preference and other adaptations to the host plant environment e.g. phenology and ovipositor length, and the correlations between the genes underlying these traits.

Even if no evidence was found that visual cues are used, an existence of visual preferences for the host plants can not be ruled out. Naturally, it is possible that the scent from the bud rather than the appearance of it is important to reveal the stage at which the bud is for the fly, and hence conclude if it is suitable for ovipositioning, since this is important for larval survival. However, it is possible visual preferences play in in ways not seen in the scope of this study. For example, all of the plant is used for male display, not only the buds, which was the part of the plant mostly visible from inside the tube. It is possible that if the study was done in a cage where the flies had seen the entire plant, the results could have been different. An important side note is also that the trials were mainly conducted inside which might have affected the outcome since the light conditions were not entirely natural even if the fluorescent lamp used provides all wavelengths of natural sunlight. However, this study could also be seen as ruling out that close-range visual preferences are important, contributing to the knowledge of the system and method development for further studies.

So in conclusion, *T. conura* is able to identify their host plant, and this indicated there is an isolating barrier between the two host plant races, although hybridization could potentially occur in sympatric zones; no signs of reinforcement was observed. Potentially, host plant preferences are strong enough to maintain the host plant races separated across the entire range examined.

It is clear that olfactory cues play an important part in finding a suitable host plant. The role of visual preferences can not be determined conclusively; a more extensive study with larger sample sizes, alternative setups for the visual trials and potentially all four categories included (allopatric *C. heterophyllum* and *C. oleraceum* flies, sympatric *C. heterophyllum* and *C. oleraceum* flies) would be of help to investigate these matters in more detail. The method for especially the visual trials was very experimental, but this study constitutes a good basis for further in-depth studies on the subject and a way of developing methods for preference trials. The degree of genetic, ecological and morphological difference between the two host plant races would still need further investigation to conclude the degree of reproductive isolation that exists between different host races of *T. conura*. It would be very interesting to identify the scent compounds underlying the observed preference, the receptors involved in sensing these compounds and the coding genetic basis and potential expression differences for the genes involved. This would shed light on whether there has been genetic differentiation in response to selection for host plant race based mate preferences. Another interesting future direction would be to test if the host plant race or the species of the plant bud the fly is raised in is the best predictor of host plant preference. This could be investigated through cross-fostering flies on the alternate plant species, and would shed light on whether the preference is genetically encoded or learnt.

REFERENCES

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. In: B.N. Petrov, F. Csaki (Eds.). Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 267-281.

Barton, N. H. 2010. What role does natural selection play in speciation? Philosophical Transactions of Royal Society B 365: 1825–1840.

Bernays, E., Graham, M. 1988. On the Evolution of Host Specificity in Phytophagous Arthropods. Ecology 69: 886–892.

Burnham, K. P. and Anderson, D. R. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer-Verlag, New York, USA.

Diegisser, T. 2005. Artbildung via Wirtsrassen bei *Tephritis conura* (Diptera: Tephritidae). Diss., Johannes Gutenberg-Universität, Mainz.

Diegisser, T., Johannesen, J., Seitz, A. 2006. The role of geographic setting on the diversification process among *Tephritis conura* (Tephritidae) host races. Heredity 96: 410-418.

Diegisser, T., Seitz, A. Johannesen, J., 2007. Morphological adaptation in host races of *Tephritis conura*. Entomologia Experimentalis et Applicata 122: 155–164.

Diegisser, T., Johannesen, J., Seitz, A. 2008. Performance of host-races of the fruit fly, *Tephritis conura* on a derived host plant, the cabbage thistle *Cirsium oleraceum:* Implications for the original host shift. Journal of Insect Science 8: 1-6.

Diegisser, T., Tritsch, C., Seitz, A., Johannesen, J. 2009. Infestation of a novel host plant by *Tephritis conura* (Diptera: Tephritidae) in northern Britain: host-range expansion or host shift?. Genetica 137: 87-97.

du Plessis, M., Johnson, S. D., Nicolson, S. W., Bruyns, P. V., Shuttleworth, A. 2018. Pollination of the "carrion flowers" of an African stapeliad (*Ceropegia mixta*: Apocynaceae): the importance of visual and scent traits for the attraction of flies. Plants Systematics and Evolution 304: 357-372.

Johannesen, J., Diegisser, T., Seitz, A. 2010. Speciation via Differential Host–Plant Use in the Tephritid Fly *Tephritis conura*. In: Glaubrecht, Matthias (Ed.). Evolution in Action: Case studies in Adaptive Radiation, Speciation and the Origin of Biodiversity. Springer-Verlag, Berlin Heidelberg, pp 239-260.

Nosil, P. 2012. Ecological Speciation. Oxford University Press. 304 pp.

Primante, C., Dötterl, S. 2010. A Syrphid Fly Uses Olfactory Cues to Find a Non-Yellow Flower. Journal of Chemical Ecology 36: 1207-1210.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. Science 323: 737–741.

Servedio, M. R., Noor, M. A. F. 2003. The Role of Reinforcement in Speciation: Theory and Data. Annual Review of Ecology, Evolution and Systematics 34: 339-364.

Svensson, G. P., Okamoto, T., Kawakita, A., Goto, R., Kato, M. 2010. Chemical ecology of obligate pollination mutualisms: testing the 'private channel' hypothesis in the Breynia–Epicephala association. New Phytologist 186: 995-1004.

Tait, C., Batra, S., Ramaswamy, S. S., Feder, J. L., Olsson, S. B. 2016. Sensory specificity and speciation: a potential neuronal pathway for host fruit odour discrimination in *Rhagoletis pomonella*. Proceedings of the Royal Society B 283: 20162101.

APPENDIX

Appendix 1. The combination of variables tested for in the visual trials, using a linear model. Note that none of the models were statistically significant.

Variables in model	F	df	p	AIC
Distribution	1.654	1, 40	0.2058	518.4439
Sex	0.3871	1, 40	0.5373	519.7409
Host plant race * Distribution	1.133	2, 39	0.3324	519.7732
Host plant race + Distribution	1.133	2, 39	0.3324	519.7732
Distribution + Sex	1.061	2, 39	0.3560	519.9207
Host plant race	0.005427	1, 40	0.9416	520.1397
Host plant race + Distribution + Sex	0.9068	3, 38	0.4468	521.2415
Host plant race + Sex	0.1930	2, 39	0.8252	521.7317
Distribution * Sex	0.7009	3, 38	0.5574	521.8833
Host plant race * Sex	0.2851	3, 38	0.8358	523.2106
Host plant race * Distribution * Sex	0.7029	5, 36	0.6249	524.2332
Distribution	1.654	1, 40	0.2058	518.4439