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Variation in sexual communication and its role in divergence of two host strains of the noctuid moth *Spodoptera frugiperda*

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Chapter 7

Discussion

1. Importance of sexual communication differences in strain divergence

The main aim of my thesis was to investigate sexual communication differences between corn- and rice-strain individuals in order to assess whether strain-specific pheromonal communication acts as prezygotic mating barrier between the two *S. frugiperda* strains. Another aim was to distinguish strain-specific variation from geographic variation and to determine the genetic basis of female pheromone differences. Based on the strain-specific variation found in signal and response, the importance of sexual communication as a prezygotic isolation barrier will be discussed and I will propose how sexual communication differences may have evolved.

1.1 Variation in the female sex pheromone signal

1.1.a Strain-specific female pheromone composition

Pheromone extractions showed that corn-strain females from Florida produced consistently lower relative amounts of the important secondary sex pheromone component Z7-12:OAc (and of Z9-12:OAc) than rice-strain females, while we found no strain-specific differences in the amounts of Z9-14:OAc and Z11-16:OAc (Chapter 2). A QTL analysis of the female sex pheromone showed that the strain-specific pheromone composition is under complex genetic control and multiple genomic regions are involved in the production of Z7-12:OAc, Z9-12:OAc, Z9-14:OAc and Z11-16:OAc (Chapter 4). Interestingly, the circadian clock protein *vriille*, which appears to be responsible for the strain-specific differences in the onset time of mating in the scotophase (Hänniger et al., 2013), mapped to one QTL for Z7-12:OAc (Chapter 4). This result suggests that two different prezygotic mating mechanisms, i.e. sex pheromone production of Z7-12:OAc (Groot et al., 2008; Chapter 2), as well as strain-specific timing of reproduction in the night

(Schöfl et al., 2009; Hänniger et al., 2013), might be genetically coupled, which could facilitate the divergence of both strains.

Although we showed that sex pheromone differences can, at least partly, be explained by genetic differences between strains (Chapter 4), the fact that we only found minor QTL ($R^2 \sim 4\% - 10\%$) suggests that other factors, like environmental variation, also influence the strain-specific sex pheromone composition. So far, it is unclear whether environmental factors like temperature, humidity, photoperiod, host plant volatiles, and/or interspecific olfactory cues can influence the sex pheromone composition of *S. frugiperda*, as it has been shown for several other moth species (McNeil and Delisle, 1989; Royer and McNeil, 1991, 1993; Delisle and Royer, 1994; Landolt and Phillips, 1997; Gemeno and Haynes, 2001; Groot et al., 2010a). Most likely, all these factors could have an effect on *S. frugiperda* females of both strains, because the pheromone composition of corn- and rice-strain females appears to be quite variable depending on different factors, as discussed below.

1.1.b Geographic variation in the female pheromone signal

In addition to strain-specific variation, geographic variation seems to be an important factor that can influence the sex pheromone of corn- and rice-strain females (Tumlinson et al., 1986; Batista-Pereira et al., 2006; Groot et al., 2008; Lima and McNeil, 2009; Chapter 2). Strain-specific differences between corn- and rice-strain females from Florida (Groot et al., 2008; Chapter 2) and Louisiana (Lima and McNeil, 2009) indicate geographic variation in Z9-14:OAc and Z11-16:OAc, whereas the critical secondary component Z7-12:OAc did not vary between these regions. In addition, geographic variation has been found in the relative amount of E7-12:OAc (Tumlinson et al., 1986; Descoins et al., 1988; Batista-Pereira et al., 2006; Groot et al., 2008). So far, it is unclear whether females

of both strains differ in their level/degree of geographic variation and thus additional research is required to disentangle variation caused by strain-specific differences from variation based on geographic differences.

1.1.c Effect of PBAN on the female pheromone composition

Analysis of pheromone extracts of laboratory corn- and rice-strain females from Florida showed that the pheromone biosynthesis activating neuropeptide (PBAN) can influence the female sex pheromone composition (Groot et al., 2008; Marr, 2009). More precisely, PBAN injection into the abdomen of corn- and rice-strain females, 1-2 hours before the pheromone gland extraction, significantly increased the relative amount of Z9-14:OAc and Z11-16:OAc in rice-strain females, and decreased the relative amount of Z7-12:OAc and Z9-12:OAc in corn- and rice-strain glands, compared to extractions in the scotophase without PBAN (Groot et al., 2008). In general, it appears that PBAN injections have greater and more consistent effects on the relative amount of minor abundant pheromone compounds (Z7-12:OAc, Z9-12:OAc) than on highly abundant pheromone compounds (Z9-14:OAc, Z11-16:OAc) (MU, unpublished data). Thus, although the use of PBAN has been described as functional to determine the native pheromone phenotype in moths like *Heliothis virescens* *H. subflexa* (Groot et al., 2005), pheromone extractions of *S. frugiperda* should be conducted only under natural conditions, i.e. within the scotophase at strain-specific female calling times (Pashley et al., 1992; Schöfl et al., 2009).

The fact that PBAN injections can change the relative amount of different pheromone compounds in corn- and rice-strain females suggests that strain-specific differences in the female pheromone composition, e.g. in the relative amount of Z7-12:OAc (Groot et al., 2008; Lima and McNeil, 2009), could be caused by strain-specific PBAN differences, if PBAN would strain-specifically interact with

pheromone biosynthesis enzymes in corn- and rice-strain females. Groot et al. (2008) suggested that PBAN could act on the reduction step of fatty acids, i.e. on the fatty acyl reductase (FAR), or could alternatively activate strain-specific $\Delta 11$ -desaturases, which still needs to be investigated.

1.1.d Intra-strain specific variation and effect of laboratory rearing

Pheromone gland extractions of corn-strain females from field Florida populations showed that females of different families exhibited significant differences in their relative amount of Z9-14:OAc, Z11-16:OAc, Z7-12:OAc and Z9-12:OAc (Marr, 2009). Thus, corn-strain females exhibit intra-strain between-family variation in their pheromone composition. An intra-strain pheromone study on rice-strain field females has not been conducted so far and therefore, we cannot state that this kind of variation also occurs in rice-strain females, although this will most likely be the case. Genetic analyses showed that the intra-strain specific variation in corn-strain females appears to be mainly determined by genetic effects, although environmental variation also seems to influence the female pheromone composition (Marr, 2009).

When we compared the pheromone composition of corn- and rice-strain females from laboratory and field population from Florida, we found significant differences within both strains between both populations, i.e. a significant increase in the amount of Z7-12:OAc, Z11-16:OAc and Z9-12:OAc, in at least one of both laboratory strains compared to field females (Chapter 2). The fact that laboratory rearing can change the pheromone composition of females has been shown for some moth species (Miller and Roelofs, 1980; Löfstedt et al., 1985; Haynes and Hunt, 1990), and is probably caused by changes in selection pressures, e.g. in the laboratory, females and males will get mated without the need to attract or be attracted. We used single pair matings to maintain our laboratory populations, but

thus forced a female to mate with one random male, which was the only mating partner available. Mating experiments with laboratory corn- and rice-strain populations showed that females are more restricted in their mate choice than males, which suggests the possible involvement of close-range male sex pheromones (Schöfl et al., 2011). Thus, the use of single pair matings might reduce possible selection pressures linked to mate choice in *S. frugiperda*, which could in turn cause a change in the female pheromone signal.

1.2 Variation in the male response to sex pheromone signals

1.2.a Habitat and geographic variation in the male response

Wind tunnel assays and field experiments in Florida showed that corn- and rice-strain males exhibit no consistent attraction to strain-specific female pheromone blends, but habitat-specific differences seem to influence the response of males to pheromone blends (Chapter 2 and 3). Most likely, host plant volatiles mediate the sexual attraction of corn- and rice-strain males, due to the fact that both strains exhibit some host-plant preferences (Pashley, 1986, 1989; Nagoshi et al., 2007; Machado et al., 2008; Groot et al., 2010b), and show differential electro-antennographic (EAG) responses to different plant volatiles (Malo et al., 2004b).

When we tested the male response towards different pheromone lures in different regions, we found that mainly corn-strain males, not rice-strain males, exhibited geographic variation in their attraction (Chapter 3). This suggests that rice-strain males exhibit a broader response spectrum towards sex pheromone blends than corn-strain males. However, due to a limited number of experiments conducted so far (Chapter 2 and 3), we cannot exclude that some environmental factors also affect the response of rice-strain males. Observations during wind tunnel experiments showed that this might be the case, because we observed that males of

both strains were highly influenced by specific experimental conditions, i.e. temperature, humidity and wind speed (Chapter 2). In addition, geographic variation in the male response to E7-12:OAc has been reported (Batista-Pereira et al., 2006; Chapter 3), although it is unclear whether males of both strains differ in their response to E7-12:OAc, and if geographic variation influences a possible strain-specific attraction to E7-12:OAc.

1.2.b Strain-specific male response to the critical secondary component

Z7-12:OAc

Trapping experiments in Florida showed that males of both strains differ significantly in their response to different amounts of Z7-12:OAc, i.e. corn-strain males are mainly attracted to 2% Z7-12:OAc, while rice-strain males mostly respond to 2-4%, but also to 10% Z7-12:OAc (Chapter 2). This strain-specific response to Z7-12:OAc is probably the most important factor that differentiates corn- and rice-strain males, and corn-strain males seem to be more restricted and specific in their response to Z7-12:OAc than rice-strain males, independent of the geographic region (Chapter 2 and 3). Differences in the olfactory pheromone receptors (ORs) on the male antennae of both strains could explain the strain-specific differences in the response to Z7-12:OAc. If corn-strain males exhibit more specifically tuned ORs for Z7-12:OAc than rice-strain males, it could explain why corn-strain males mainly respond to 2% Z7-12:OAc and rice-strain males to 2% up to 10% Z7-12:OAc. Genetic analyses of *S. frugiperda* field populations showed that corn-strain populations are genetically much more homogenous than rice-strain populations (Juárez et al. 2013), which could be correlated to possible strain-specific differences in ORs responding to Z7-12:OAc.

1.3 Strain-specific sexual communication as weak prezygotic isolation barrier

Although we found consistent strain-specific differences in the female pheromone composition of both strains, corn- and rice-strain males were not exclusively attracted to females of their own strain in Florida (Meagher and Nagoshi, 2013; Chapter 2). Similarly, Pashley et al. (1992) showed that corn- and rice-strain males from Louisiana were not strain-specifically attracted to traps baited with virgin corn- and rice-strain females. These data indicate that strain-specific sexual communication is a weak prezygotic isolation barrier and is unlikely to be a major force in the divergence of both *S. frugiperda* strains, at least not in North America (Florida and Louisiana).

On the other hand, geographic variation influences the male response (Chapter 3), which could mean that strain-specific sexual communication might drive divergence of both strains in other regions than North America, i.e. in South America. Two different field studies suggest that the role of E7-12:OAc is especially important in regions in South America, i.e. in Brazil (Batista-Pereira et al., 2006), and Peru (Chapter 3). If rice-strain males in South America would be attracted to E- and Z-7-12:OAc, while corn-strain only respond to Z7-12:OAc, assortative mating could occur. However, so far we can only speculate that sexual communication might contribute to the divergence of the two strains in South America.

1.4 Evolution of strain-specific sexual communication differences

Genetic analyses of *S. frugiperda* field populations showed that corn-strain populations are genetically much more homogenous than rice-strain populations, suggesting that the corn-strain went through a bottleneck (Juárez et al. 2013). In

Chapter 6, we proposed an evolutionary scenario with the rice-strain as ancestral strain and the corn-strain as derived strain that arose from a few individuals. A corn-strain specific genetic homogeneity could explain why corn-strain males are more restricted in their response to a female sex pheromone than rice-strain males (Chapter 2 and 3). Interestingly, we found a congruence between the production of and the response to Z7-12:OAc in corn-strain populations from Florida (Chapter 2). These data suggest that a change in the pheromone production of the ancient rice-strain population might have been fixed due to bottleneck effects, which in turn has led to a small change in the sexual communication system of the derived corn-strain population. QTL analysis of the female sex pheromone of *S. frugiperda* showed that multiple genomic regions are involved in the production of strain-specific pheromone blends (Chapter 4), which indicates that changes in the pheromone composition might have occurred in multiple minor steps. This would explain the fact that a total of 10 minor QTLs explained the strain-specific pheromone differences (Chapter 4).

With all our experiments, we focused on 5 different compounds that were found in the pheromone glands of corn- and rice-strain females, i.e. Z9-14:OAc, Z7-12:OAc, E7-12:OAc, Z11-16:OAc and Z9-12:OAc (Chapter 2-4). Based on all results, I will now speculate how strong or weak selection pressures might act on these 5 different compounds.

Z9-14:OAc. The major pheromone component Z9-14:OAc seems to be under strong selection, because it has been found as main abundant component in glands of both strains in different regions (Tumlinson et al., 1986; Batista-Pereira et al., 2006; Groot et al., 2008; Lima and McNeil, 2009; Chapter 2), and it is necessary for male attraction in the field (Tumlinson et al., 1986; Andrade et al., 2000; Batista-Pereira et al., 2006; Chapter 2 and 3). Due to the fact that corn- and rice-strain females from Florida show no strain-specific differences in their relative

amount of Z9-14:OAc (Chapter 2), we did not conduct a Z9-14:OAc dose-response experiment. However, Lima and McNeil (2009) reported that corn- strain females from Louisiana produce higher amounts of Z9-14:OAc than rice-strain females, and thus male response to Z9-14:OAc might differ between strains from this region.

Z7-12:OAc. Corn- and rice-strain females exhibit significant differences in their relative amount of Z7-12:OAc (Groot et al., 2008; Lima and McNeil, 2009), and males show strain-specific responses to different doses of Z7-12:OAc (Chapter 2 and 3). Thus, it seems that the critical secondary sex pheromone component Z7-12:OAc is under relatively strong strain-specific selection. Interestingly, our QTL analysis suggests that the sex pheromone differences in Z7-12:OAc are genetically linked to strain-specific timing of mating via *vrille* (Chapter 4). Such a genetic linkage could be achieved via a cis-regulatory element that influences both the expression of *vrille* and pheromone biosynthesis enzymes like desaturases or PBAN.

E7-12:OAc. Female production of and male response to E7-12:OAc varies significantly between regions (Tumlinson et al., 1986; Batista-Pereira et al., 2006; Groot et al., 2008; Lima and McNeil, 2009; Chapter 3), and E7-12:OAc appears to be especially important for the attraction of males in western South America. Thus, selection pressures acting on E7-12:OAc production and response could be strong in some regions, while in other regions this component is not produced by females and therefore unnecessary for male attraction .

Z11-16:OAc and Z9-12:OAc. Our trapping experiments showed that Z11-16:OAc has only a small, if any, effect on the response of corn- and rice-strain males (Chapter 2 and 3). This suggests that Z11-16:OAc is under weak selection, if at all, which is supported by the fact that seven genomic regions are involved in the production of Z11-16:OAc (Chapter 4). Our experiments also evidence that Z9-12:OAc is not required for attraction of corn- and rice-strain males (Chapter 2),

which suggests minor or no selection on the relative amount of Z9-12:OAc in the pheromone blend.

1.5 Importance of different reproductive mating barriers

Our and many other studies have shown that both *S. frugiperda* strains exhibit genetic and behavioral differences, and so far three possible prezygotic mating barriers, as well as one postzygotic isolation mechanism, have been described (Pashley et al., 1992; Schöfl et al., 2009; Groot et al., 2010b; Juárez et al., 2012). Although both strains were first considered to exhibit strong host-associations, more recent studies show that host associations are not as clear-cut as previously thought, as both strains can often be found together in many habitats (Pashley, 1989; Meagher and Gallo-Meagher, 2003; Nagoshi et al., 2007; Juárez et al., 2012; Chapter 6). Thus, reproductive isolation solely due to host plant differences seems to be a weak prezygotic isolation mechanism between the two strains. Similarly, strain-specific sexual communication does not appear to be a strong prezygotic mating barrier between the strains (Pashley et al., 1992; Meagher and Nagoshi, 2013; Chapter 2). Instead, consistent strain-specific, geographically independent, differences in the timing of reproductive behaviors (female/male calling, copulation, oviposition) seem to be the strongest prezygotic mating barrier that drives divergence between strains (Pashley et al., 1992; Schöfl et al., 2009; Schöfl et al., 2011; Hänniger et al., 2013). Even though strain-specific timing of mating is probably the strongest of all prezygotic barriers (Figure 1), strain-specific mating times do overlap to some degree (Schöfl et al., 2009), and this barrier alone is most likely also not sufficient to completely prevent hybridization (Schöfl et al., 2009, 2011).

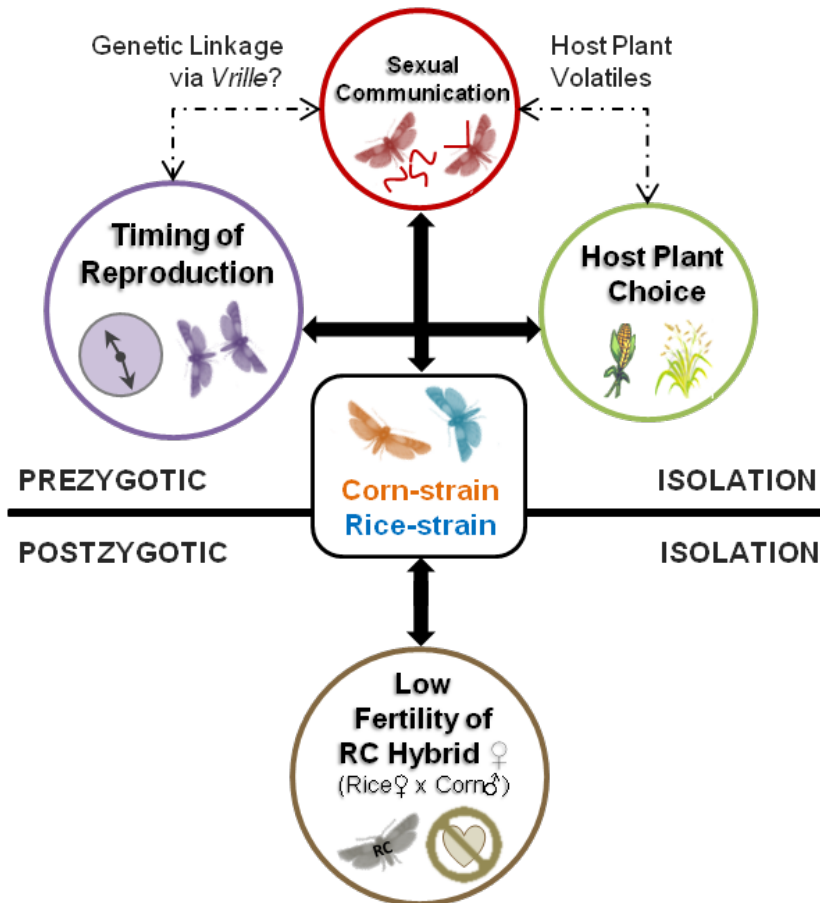


Figure 1. Proposed interactions and importance of different pre- and post-zygotic isolation barriers involved in the divergence of *S. frugiperda* corn- and rice- strains. The size of each circle represents the strength of an isolation barrier, i.e. large circles symbolize very important mechanisms and small circles less important ones.

Besides prezygotic isolation, postzygotic isolation seems to be important in the divergence of both strains, i.e. the partial sterility of RC (rice-strain♀ x corn-strain♂) hybrid females (Groot et al., 2010b; Kost et al. unpublished; Figure 1). This one-sided hybrid mating incompatibility appears to be a strong postzygotic

barrier, which probably acts together with the prezygotic isolation barriers to reduce gene flow between strains (Groot et al., 2010b; Figure 1). Together, all data suggest that both *S. frugiperda* strains exhibit several incomplete reproductive isolation barriers that prevent both strains from merging into one mixed population (Groot et al., 2010b). Therefore, both *S. frugiperda* strains seem to have acquired some elements of species rank, but exhibit incomplete reproductive isolating mechanisms and are still able to hybridize. Alternatively, it could be possible that geographic isolation caused divergence and partial reproductive isolation mechanisms developed, while now ongoing hybridization is merging both strains.

2. Sexual communication and pest management of *Spodoptera frugiperda*

The strain-specific differences that we found in the sexual communication system of *S. frugiperda* corn- and rice-strain individuals may be useful to improve different pest management strategies and/or to optimize the control of this agricultural pest species. Based on the results of Chapter 2 and 3, I recommend a general pheromone blend to monitor both strains independent of geographic and strain-specific variation, and try to assess whether it is possible to reduce *S. frugiperda* populations in the field via the use of mating disruption.

2.1 Monitoring

Although we found strain-specific differences in the attraction of males to different amounts of Z7-12:OAc, responses of both strains do overlap to some degree. Also, corn- and rice-strain males from different regions show no consistent strain-specific attraction to female pheromone blends in the field (Chapter 2 and 3). Thus, the use of strain-specific pheromone lures to trap only *S. frugiperda* corn- or rice-

strain males is probably not doable, meaning that the same pheromone lures can be (more or less efficiently) used for both strains.

The results of our male-trapping experiments showed that two pheromone components, i.e. Z9-14:OAc and Z7-12:OAc, are required for the attraction of corn- and rice-strain males in different regions in North and South America (Chapter 2 and 3). Thus, binary blends containing Z9-14:OAc and Z7-12:OAc are probably the best choice to monitor *S. frugiperda*. More precisely, blends containing 100% Z9-14:OAc (300µg) and 2% Z7-12:OAc (6µg) could be most effective for monitoring of both strains within different habitats, because males of both strains show an equally high response to such blends (Chapter 2 and 3). Higher amounts of Z7-12:OAc, i.e. 5% (Andrade et al., 2000) or 10% (Chapter 2 and 3), can reduce the response of *S. frugiperda* and should therefore be avoided when monitoring this species.

An universal trap design that can be used for our monitoring blend (100% Z9-14:OAc + 2% Z7-12:OAc) cannot be concluded from our experiments, because we used the same green-yellow-white bucket traps in all experiments (Chapter 2 and 3). Trapping experiments of *S. frugiperda* showed that male attraction can vary depending on different factors like color and shape of the pheromone traps, and also geographic variation seems to be involved in this variation (Mitchell et al., 1989; Meagher and Mitchell, 2001; Malo et al., 2004a). In Florida, green-yellow-white bucket traps seem to be most efficient to catch *S. frugiperda* (Meagher and Mitchell, 2001), while in Mexico cone traps are more attractive than bucket traps (Malo et al., 2004a). To what extent *S. frugiperda* males can be repelled from a pheromone trap due to visual cues is so far unknown and still needs to be investigated.

2.2 Control via mating disruption

The saturation of the environment with synthetic sex pheromone, i.e. mating disruption, is used to confuse males with the aim to reduce/delay matings with females, because the location of conspecific females is masked by the smell of synthetic pheromones (Taschenberg and Roelofs, 1978; Witzgall et al., 2008; Cocco et al., 2013; Trematerra and Spina, 2013). The use of mating disruption has been shown to be effective in decreasing pest populations of moth species like *Cydia pomonella*, *Grapholita lobarzewskii* or *Lobesia botrana* (Witzgall et al., 2008; Gambon et al., 2009; Ioriatti et al., 2011). In *S. frugiperda*, synthetic binary pheromone blends containing 100% Z9-14:OAc and 2% Z7-12:OAc could be used as blends in mating disruption, due to the consistently high and geographically independent attraction of corn- and rice-strain males to this blend (Chapter 2 and 3). Interestingly, high amounts (10%) of Z7-12:OAc can reduce the attraction of corn- and rice-strain males (Chapter 2 and 3), which suggests that saturation of a field with high dosages of solely Z7-12:OAc might confuse males of both strains enough to lead to a reduction of matings. If this would be case, the costs of synthetic pheromone dispensers could be drastically reduced when only Z7-12:OAc is used, instead of Z9-14:OAc and Z7-12:OAc.

One great advantage of mating disruption, in contrast to trap-and-kill methods, is that it does not require any pheromone traps or killing agents because males are solely repelled from a field. Besides the fact that this makes the mating disruption cheaper, one does not need to worry about possible geographic variation in the trap color and design, which was described above. However, a major drawback of this method is that it works most efficiently when population densities are low and field sites isolated (Witzgall et al., 2008). The fact that *S. frugiperda* is a generalist species (Pashley, 1988), that can infest large areas of agricultural land (Luginbill, 1928; Sparks, 1979, 1986), suggests that this might lead to problems in the

efficiency of this method. Nevertheless, I think it will be worthwhile to develop the use of mating disruption to reduce the density of and damage caused by *S. frugiperda* corn- and rice-strain populations in agricultural crops.

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