Evolutionary Ecology Research, 2004, **6**: 927–935

Divergence revealed by population crosses in the red flour beetle *Tribolium castaneum*

Fathi A. Attia‡ and Tom Tregenza*

Ecology and Evolution Group, School of Biology, University of Leeds, Leeds LS2 9JT, UK

ABSTRACT

There is growing interest in the potential for population divergence (and hence speciation) to be driven by co-evolutionary arms races due to conflicts of interest between the sexes over matings and investment in offspring. It has been suggested that the signature of sexually antagonistic co-evolution may be revealed in crosses between populations through females showing the weakest response to males from their own population compared with males from other populations. The rationale behind this prediction is that females will not have been able to evolve counter-adaptations to manipulative signals from males with which they have not co-evolved. Recent theoretical treatments suggest that this prediction is not strictly exclusive to the sexual conflict theory, but it remains the case that population crosses can provide insights into the evolution of mate choice within populations. We describe crosses between six populations of the red flour beetle *Tribolium castaneum*. Although successful matings are no more or less likely between populations compared to within populations, females do increase their oviposition rate in response to males from other populations, relative to males from their own population. Our results are therefore consistent with the proposition that sexual conflict has driven population divergence in this species. However, we argue that the available evidence is more supportive of the hypothesis that increased female investment in response to males from other populations is a side-effect of inbreeding avoidance within populations.

Keywords: cryptic female choice, differential investment, heterosis, inbreeding, outbreeding, sexual conflict, speciation.

INTRODUCTION

The potential for sexual selection to drive speciation has traditionally been considered from the point of view of the potential for mate recognition and choice to create pre-mating isolation between populations. However, there is growing interest in the potential for divergence between populations to be manifest in processes occurring after mating (Rice, 1997; Parker and Partridge, 1998; Howard, 1999). Males may be able to manipulate female investment in their offspring (sexual conflict; Parker, 1979), or females may adjust

---

* Author to whom all correspondence should be addressed. e-mail: t.tregenza@leeds.ac.uk

‡ *Present address*: Department of Zoology, Faculty of Science, University Garyounis, PO Box 17074, Benghazi, Libya.

their own investment according to the quality of their mate (differential allocation; Burley, 1986). If either of these types of post-copulatory effects occurs, then there is the potential for divergence between populations in the signal–receptor systems that underlie such processes. Divergence between populations may tend to promote isolation if cross-population matings produce fewer offspring than intra-population matings, or may tend to retard isolation if matings between populations tend to produce more offspring than those within populations (Parker and Partridge, 1998). To date, relatively few studies have examined the effects of crosses between populations on female fecundity, so it is difficult to establish whether there is any general pattern. Recent interest has focused on the potential for sexual conflict to drive rapid co-evolution between the sexes, with males attempting to manipulate females into using their sperm and investing resources in their offspring and females attempting to avoid manipulation. This has motivated several studies in which crosses between populations have revealed differences in female fecundity post-mating according to the population of both the male and female (Clark *et al.*, 1999; Andres and Arnqvist, 2001; Hosken *et al.*, 2002; Nilsson *et al.*, 2002, 2003). Here we describe a study examining crosses between six populations of the flour beetle *Tribolium castaneum*, examining post-copulatory female fecundity and attempting to understand the high level of copulations that do not result in sperm transfer.

Tribolium castaneum is a worldwide pest of stored products. Females mate multiply: up to 15 times per hour (Sokoloff, 1977), even though after a single mating they can continue to fertilize eggs for up to 140 days (Bloch-Qazi *et al.*, 1996). *Tribolium castaneum* has been observed to show high levels of mating failures (copulations that do not result in sperm transfer) (Bloch-Qazi *et al.*, 1996). Insight into the reasons for these failed matings would be valuable in understanding female polyandry in this and other species.

For successful copulation to occur in *Tribolium*, the female must lower her last sternite to allow intromission (Lewis and Iannini, 1995). Bloch-Qazi *et al.* (1996) found that in 20 out of 130 *T. castaneum* copulations, no spermatophore was transferred and no sperm were observed in any region of the female reproductive tract. Bloch-Qazi *et al.* (1998) found that both anaesthetized females and dead females had fewer stored sperm after mating than unanaesthetized females, suggesting that females play an active role in moving sperm from the site of deposition into storage in the spermatheca. Hence mating failures may be due to failure of intromission or failure by females to store sperm. Either way, one explanation for the high incidence of mating failures seen in this species is that there is a conflict of interests between mates, and that females are effectively rejecting sperm from certain males, even when they have no sperm already stored. Such conflicts are predicted to drive rapid co-evolutionary arms races between the sexes, with males evolving adaptations to increase sperm transfer success, and females evolving counter-adaptations to continue to choose between males through rejection of sperm. If such a process is ongoing in *T. castaneum*, we might expect to see either increased or decreased numbers of successful matings in crosses between populations relative to pairings within populations.

A second area where we may expect co-evolution between the sexes is in signals from males used by females to adjust their investment in eggs or their oviposition rate. This may be characterized as a conflict where males are attempting to manipulate females into oviposition at a higher than optimal rate, or it may be a more conventional sexual selection scenario where females increase investment in eggs when they perceive they have mated with a genetically superior male (differential allocation) or a male with whom they are genetically compatible (Zeh and Zeh, 1996). Both processes involve conflict, in the former between females and all mates, and in the latter between females and non-preferred mates. Either process could lead to co-evolution between male signals and female responses. If there is a conflict between the sexes with males attempting to increase female egg-laying rate, we might expect lower egg-laying rates in crosses within populations because females may be better able to resist males with which they have co-evolved. A statistical interaction *per se* is not evidence for sexual conflict co-evolution, since other forms of sexual selection, such as good genes processes, also predict interactions across populations (Panhuis *et al.*, 2001); however, other theories do not predict that females will be least willing to mate and will invest least in reproduction in response to males from their own population (although they don't rule out this possibility). In general, the theory surrounding sexual conflict co-evolution is in the early stages of development, and unequivocal predictions are lacking (Chapman *et al.*, 2003). However, there is no doubt that a body of empirical work that can indicate whether there is a general pattern of lower rates of matings, fertilization and subsequent reproductive investment by females paired with males from their own population will inform theoretical development in this field.

In a previous study, Nilsson *et al.* (2002) investigated crosses between three strains of *T. castaneum* (nine crosses) and found an interaction between male and female strain affecting female reproductive rate, although there was no clear pattern of response to males of the same strain as opposed to males of a different strain. Our study involves crosses between six populations (36 crosses), with the aim of increasing the power of the test of the prediction that females may show either maximal or minimal responses to males from their own strain. Additionally, we explicitly examine success in sperm transfer, a major component of male reproductive success in this species that has not previously been examined.

METHODS

Six strains of *T. castaneum* were used, all of which have been isolated from one another for hundreds of generations. These strains were: Ga1 (wild type), b (black body), Rd (reindeer antennae), Chr-E (charcoal body colour), Det-43 (divergent elytra tips) and Oz (collected in Australia). The first four strains were all supplied by R. Beeman, US Grain Marketing and Production Research Center. All populations have been kept under very similar environmental conditions, so it is unlikely that there will be differences in the strength of natural selection on sexually selected traits such as female receptors and male signals. The marker strains used are all single-locus markers that are neutral or only mildly deleterious, and that have been back-crossed into their ancestral populations, hence they are not expected to suffer from greater homozygosity than other populations, all of which have been maintained in similar conditions for hundreds of generations.

A virgin male and female, aged 7–21 days post-eclosion, were placed into a 2×2 cm cubic arena with filter paper on the floor to provide traction. The pair was observed until a mating took place (typically within a few minutes). The time to mating and the duration of the mating were recorded. Only copulations lasting longer than 40 s were included in subsequent analyses, as shorter interactions may not allow sperm transfer to occur. After copulation, the female was placed in a 100-ml pot containing 30 ml fine organic flour for 7 days at 30°C and 65% relative humidity to lay eggs. The presence of larvae in these pots after 2 weeks was used to confirm that successful sperm transfer has occurred during the copulation. Forty days after the last day of oviposition, offspring were counted (providing ample time for all fertile eggs to hatch and reach adulthood). Males were killed by freezing immediately after mating, females were killed after oviposition and the size of both sexes was measured using a microscope with graticule.

All reciprocal crosses were carried out between strains with 10 replicates per cross, giving 360 crosses in total. Parametric statistics were only employed after confirming the data were normally distributed.

RESULTS

Of a total of 360 matings, 250 resulted in the female laying fertile eggs (69.4%). The actual numbers of successful matings in each cross can be seen in Fig. 1. Logit log-linear analysis indicates that there was no effect of either male or female strain on sperm transfer (male strain, $\chi^2 = 26.2$, d.f. = 30, $P = 0.66$; female strain, $\chi^2 = 32.7$, d.f. = 30, $P = 0.34$). There was also no interaction between strains affecting the success or failure of matings ($\chi^2 = 18.8$, d.f. = 25, $P = 0.81$). However, this type of analysis has low statistical power. A more powerful way of addressing the specific hypothesis that there will be differences (but no direction) in the number of successful matings in intra-population crosses relative to inter-population crosses is to compare directly the numbers of successful and unsuccessful matings in these two groups. A contingency χ^2 test of this specific hypothesis also fails to find any support ($\chi^2 = 0.67$, d.f. = 1, $P = 0.41$), with mating failures in 21/60 (35%) of intra-population copulations and 89/300 (30%) of crosses between populations. Matings where successful sperm transfer occurred did not differ in duration from those where no sperm were transferred ($t = 0.38$, d.f. = 358, $P = 0.70$). The average duration of successful matings was 136.2 ± 7.24 s and for unsuccessful matings 150.2 ± 11.8 s (bearing in mind that copulations were only considered to have occurred when pairings lasted more than 40 s).

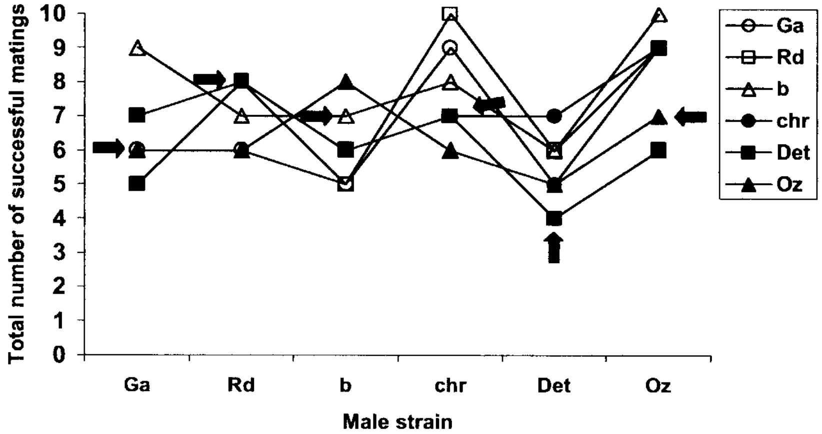

Fig. 1. The number of matings where sperm transfer was successful out of 10 copulations between strains (360 matings in total). Arrows indicate intra-strain crosses. Lines are drawn only as an aid to interpretation.

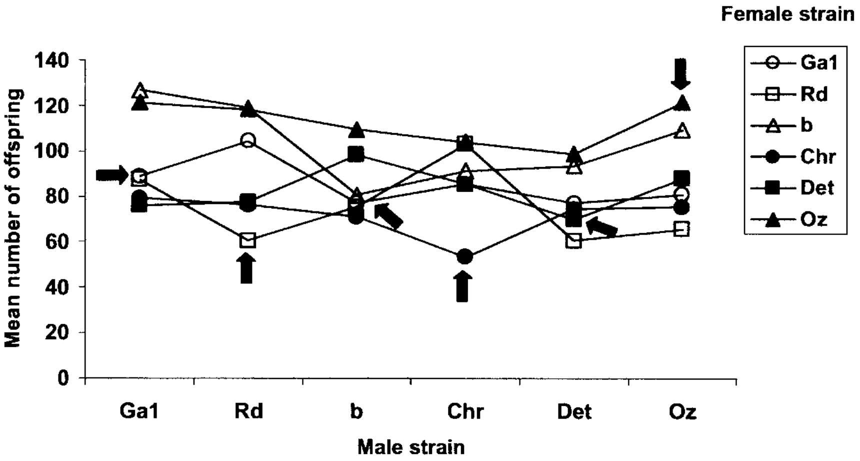

Fig. 2. The mean number of offspring produced from eggs laid in the week following mating by 10 females from each of the five different strains with males from all strains in all reciprocal combinations (360 females) (only using data where successful sperm transfer occurred). Arrows indicate intra-strain crosses. Error bars are omitted as they overlap completely with one another; lines are drawn only as an aid to interpretation.

The number of offspring produced by females mating to males of the different strains (omitting females that failed to produce any offspring) can be seen in Fig. 2. Analysis of variance indicates that there was a significant effect of the interaction between strains of both sexes on the number of adult offspring the female subsequently produced (female × male strain: $F_{25,214} = 2.04$, $P = 0.004$; female strain, $F_{5,214} = 18.2$, $P < 0.001$; male strain, $F_{5,214} = 2.33$, $P = 0.044$). There were differences in size between strains, but when male and female size were included in our analyses, they revealed an effect of female size on number of adult offspring but no effect of male size, and no change in the significant interaction between male and female strain affecting offspring production (female × male strain, $F_{25,212} = 2.17$, $P = 0.002$; female size, $F_{1,212} = 10.9$, $P = 0.001$; male size, $F_{1,212} = 1.45$, $P = 0.23$). The age of beetles of either sex did not affect egg production [adding age since eclosion into our model as a covariate (female age, $F_{1,212} = 0.95$, $P = 0.33$; male age, $F_{1,212} = 0.56$, $P = 0.46$)].

Ignoring specific populations and simply comparing the offspring production of females following inter- and intra-population crosses, shows that inter-population crosses ($n = 39$) result in higher offspring production (mean ± standard error = 90.9 ± 1.93) than do within-population crosses ($n = 211$, mean ± standard error = 79.1 ± 5.46) ($t = -2.32$, d.f. = 248, $P = 0.021$).

DISCUSSION

Mating failures

We found that a large proportion of matings (31.6%) did not result in the female laying fertile eggs. This is higher than the 15.4% found in a previous study (Bloch-Qazi *et al.*, 1996), although in that study mating success was measured as sperm being found in the female. There may be reasons why females that have received sperm subsequently reject it or fail to lay any fertilized eggs. Any such effects would increase the number of apparent mating failures in our study compared with the earlier study. We did not find any effect of population on the rate of mating failure or any evidence for differences in failure rate in intra-population copulations as opposed to inter-population copulations. This suggests that the very high frequency of mating failures seen in this species is not due to a co-evolutionary interaction between the sexes where females are attempting to avoid being fertilized by certain males. This hypothesis is intrinsically difficult to test, as each mating can only be categorized as failing or succeeding. Hence the lack of an effect in this study cannot be considered strong evidence that mating success is not affected by the population of the male and female, but it does indicate that any such effects are not very large.

Reproductive rate

The second parameter studied was offspring production in the week immediately following matings. A week is a biologically significant period of time because the generation time is only a few weeks and because it is likely that females will typically re-mate within a few days in natural situations. Four female strains (Rd, b, Chr and Det) out of the six we studied produced fewer offspring when they mated to males from their own strain than when they mated to males from any of the other six strains (using all data, including females that failed to lay any fertile eggs). A comparison of the average number of offspring produced by homogamic and heterogamic crosses showed that the mean number of offspring produced by homogamic crosses was significantly less than the mean number of offspring produced by heterogamic crosses. It is possible that this might be because of increased larval survival in crosses between populations compared to within populations; however, this is unlikely, since the strains used show strong inbreeding depression in full-sib crosses (personal observation), limiting the potential for fitness benefits of outbreeding. There is evidence that males may be able to stimulate female oviposition; Lewis and Austad (1994) found that polyandrous females laid more eggs in the 24 hours post-mating than monandrous females. This effect is unlikely to have been due to sperm limitation in monandrous females, since a singly mated female can lay fertilized eggs for several weeks (Bloch-Qazi *et al.*, 1996). Our analysis reveals that the number of offspring produced by females in the week following a single mating depended on an interaction between the female's genotype and that of her mate, and that overall females mating to males from their own population produced fewer offspring in the following week than those mating to males from other populations. When an interaction effect is significant, interpretation of main effects is problematic. Nonetheless, the much larger effect of female population on oviposition rate, relative to male population, is as would be expected, since females are more likely to affect their own oviposition rate than are their mates.

Our results are consistent with those of Nilsson *et al.* (2002), who found that in crosses between three populations, female *T. castaneum* differed in lifespan, reproductive rate and lifetime fecundity according to interactions between the genotypes (populations) of males and females. This interaction was complex and depended on female mating frequency. Nilsson *et al.* argue that female reproductive rate and lifetime offspring production were significantly lower with males with which females had co-evolved than with any other genotype. However, differences between male genotypes in their effect on females were significant in only two of three female genotypes in the high mating frequency treatment and in no case in the low mating frequency treatment. In the third female genotype of the high frequency treatments and all genotypes of the low mating frequency treatments, males did not produce less offspring when they mated to females with which they have co-evolved. More recently, Nilsson *et al.* (2003) studied the effect of genotype on fertilization success in three different wild-type genotypes of *T. castaneum* (the same strains used in Nilsson *et al.*, 2002). They conducted a double mating experiment in which all females mated twice, once to a wild-type male and once to a phenotypic marker (black) male in all possible reciprocal combinations. They examined female refractory period and the fertilization success of the first and second male to mate. A successful mating was defined as lasting more than 35 s, although such copulations do not always involve successful sperm transfer. Therefore, their measures of post-mating success include cases in which one male failed to transfer sperm. After the female mated, offspring were collected over two consecutive weeks. They found that female genotypes differed in the proportion of offspring sired by the first male to mate (P_1) and the overall interaction showed that the females showed the shortest average refractory period when previously mated to males of their own genotype. Male and female genotypes also interacted strongly in influencing the proportion of offspring sired by the second male (P_2) – that is, high P_2 in one genotype depended on the genotype of his mate. Nilsson *et al.* (2003) concluded that female genotype influenced male success in both male offence (P_2) and defence (P_1), suggesting that cryptic female choice is important in shaping male fertilization success in *T. castaneum*, and that females may have co-evolved resistance to post-copulatory manipulations of males with which they are co-evolved.

Our study supports the findings of Nilsson *et al.* (2002, 2003), and studies of *Musca domsetica* (Andres and Arnqvist, 2001) and *Scathophaga stercoraria* (Hosken *et al.*, 2002), all of which found tendencies for lower mating rates or lower reproductive investment by females paired with homogamic males. However, although sexual conflict co-evolution may result in situations where females are less stimulated to mate and invest in offspring in response to males from their own strain than to males with which they have not co-evolved, it is not clear that this will always be the case (Rowe *et al.*, 2003). A possible alternative explanation for the observation that females increase reproductive investment following matings to heterogamic males across species is that this could be the signature, not of sexual conflict arms races, but of female adaptations to avoid inbreeding (Clark *et al.*, 1999). Reduced fitness of offspring from matings with relatives creates selection for adaptations that decrease female investment in such offspring. This could lead to females reducing reproductive investment in genetically similar males compared to more divergent males as a method of avoiding inbreeding (Tregenza and Wedell, 2000). A side-effect of such adaptations might be increased female reproductive investment following matings to heterogamic males. This explanation is arguably more consistent with the situation in *Tribolium*, where the available evidence suggests that females benefit from the higher oviposition rate that non-population males induce. Females increase their lifetime reproductive success when mating with non-population males and when mating more frequently (Nilsson *et al.*, 2002), which is the opposite of what would be expected under the conflict hypothesis. Similar to the inbreeding avoidance hypothesis is the possibility that females can take advantage of heterosis through preferentially investing in offspring of males from other populations (Bateson, 1983). This hypothesis of selection for mate preference in relation to outbreeding differs crucially from the inbreeding avoidance hypothesis, which predicts preference for outbred males as an unselected side-effect of inbreeding avoidance. It is difficult to see how a strategy for preferring non-population mates can evolve, since it requires frequent matings between populations, which in itself will erode genetic differentiation (Tregenza and Wedell, 2000). Our finding that females invest more in reproduction following matings to males from populations other than their own is consistent with the idea that there are conflicts of interest between the sexes in *T. castaneum*, but it is clear that the system would benefit from further investigation, particularly into the nature of the signals and receptors used by the two sexes.

ACKNOWLEDGEMENTS

We thank R. Beeman and T. Evans for supplying *Tribolium* stocks and Göran Arnqvist, Locke Rowe, Matt Gage, Roger Butlin and an anonymous reviewer for comments on the manuscript. F.A.A. was supported by a scholarship from the University of Garyounis, Benghazi, Libya. T.T. was supported by a Royal Society Fellowship.

REFERENCES

Andres, J.A. and Arnqvist, G. 2001. Genetic divergence of the seminal signal–receptor system in houseflies: the footprints of sexually antagonistic coevolution? *Proc. R. Soc. Lond. B*, **268**: 399–405.

Bateson, P. 1983. Optimal outbreeding. In *Mate Choice* (P. Bateson, ed.), pp. 257–277. Cambridge: Cambridge University Press.

Bloch-Qazi, M.C., Herbeck, J.T. and Lewis, S.M. 1996. Mechanisms of sperm transfer and storage in the red flour beetle (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.*, **89**: 892–897.

Bloch-Qazi, M.C., Aprille, J.R. and Lewis, S.M. 1998. Female role in sperm storage in the red flour beetle, *Tribolium castaneum*. *Comp. Biochem. Physiol. A: Molec. Integr. Physiol.*, **120**: 641–647.

Burley, N. 1986. Sexual selection for aesthetic traits in species with biparental care. *Am. Nat.*, **127**: 415–445.

Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L. 2003. Sexual conflict. *Trends Ecol. Evol.*, **18**: 41–47.

Clark, A.G., Begun, D.J. and Prout, T. 1999. Female × male interactions in *Drosophila* sperm competition. *Science*, **283**: 217–220.

Hosken, D.J., Blanckenhorn, W.U. and Garner, T.W.J. 2002. Heteropopulation males have a fertilization advantage during sperm competition in the yellow dung fly (*Scathophaga stercoraria*). *Proc. R. Soc. Lond. B*, **269**: 1701–1707.

Howard, D.J. 1999. Conspecific sperm and pollen precedence and speciation. *Annu. Rev. Ecol. Syst.*, **30**: 109–132.

Lewis, S.M. and Austad, S.N. 1994. Sexual selection in flour beetles – the relationship between sperm precedence and male olfactory attractiveness. *Behav. Ecol.*, **5**: 219–224.

Lewis, S.M. and Iannini, J. 1995. Fitness consequences of differences in male mating-behavior in relation to female reproductive status in flour beetles. *Anim. Behav.*, **50**: 1157–1160.

Nilsson, T., Fricke, C. and Arnqvist, G. 2002. Patterns of divergence in the effects of mating on female reproductive performance in flour beetles. *Evolution*, **56**: 111–120.

Nilsson, T., Fricke, C. and Arnqvist, G. 2003. The effects of male and female genotype on variance in male fertilization success in the red flour beetle (*Tribolium castaneum*). *Behav. Ecol. Sociobiol.*, **53**: 227–233.

Panhuis, T.M., Butlin, R., Zuk, M. and Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.*, **16**: 364–371.

Parker, G.A. 1979. Sexual selection and sexual conflict. In *Sexual Selection and Reproductive Competition in Insects* (M.S. Blum and A.N. Blum, eds.), pp. 123–166. London: Academic Press.

Parker, G.A. and Partridge, L. 1998. Sexual conflict and speciation. *Phil. Trans. R. Soc. Lond. B*, **353**: 261–274.

Rice, W.R. 1997. Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In *Endless Forms* (S.H. Berlocher, ed.), pp. 261–270. New York: Oxford University Press.

Rowe, L., Cameron, E. and Day, T. 2003. Detecting sexually antagonistic coevolution with population crosses. *Proc. R. Soc. Lond. B*, *Biol. Sci.*, **270**: 2009–2016.

Sokoloff, A. 1977. *The Biology of Tribolium with Special Emphasis on Genetic Aspects*, Vol. 1. Oxford: Oxford University Press.

Tregenza, T. and Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage. *Mol. Ecol.*, **9**: 1013–1027.

Zeh, J.A. and Zeh, D.W. 1996. The evolution of polyandry, I: Intragenomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B*, **263**: 1711–1717.