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A new strategy of heterosis research in mice – approach and results on chromosome 19

Dedicated to Prof. Dr. Dr. h.c. Göttfried Leuthold on the occasion of his 65th birthday

Summary

The following steps were performed to analyse heterosis and QTL effects in litter size of mice: intercross of mouse inbred strains C57BL/6J and Balb/cJ in order to produce a F₂ generation with 948 female animals; selection of trait groups with extreme high (13 offspring) and extreme low litter size (5 offspring); typing of 56 microsatellites with an average distance of 32 cM; detection of different chromosome regions with associations to heterosis in litter size. Chromosome 19 was associated to heterosis in litter size. Additional animals with extreme high and low litter sizes were then typed for four DNA markers on chromosome 19 and used for QTL mapping. A QTL was identified for litter size in segment D19Mit28 - D19Mit99 with a maximum at 15 cM ($p \leq 0.05$). The QTL explains about 11 % of the phenotypic variance in the F₂ generation. With a degree of dominance of 4.09 the QTL shows that superdominance can explain heterosis in litter size.

Key words: heterosis, fertility, mouse, DNA marker, QTL

Zusammenfassung

Titel der Arbeit: Eine neue Strategie der Heterosis-Forschung an Mäusen - methodischer Ansatz und Ergebnisse auf Chromosom 19

Die folgenden Schritte wurden zur Analyse der Heterosis und zum Nachweis von QTL-Effekten für die Wurfgröße bei der Maus durchgeführt: Intercross der Mäuse-Inzuchtstämme C57BL/6J und Balb/cJ zur Bildung einer F₂-Generation mit 948 weiblichen Tieren; Selektion von Tieren verschiedener Leistungsgruppen mit extrem hoher und niedriger Wurfgröße (13 bzw. 5 Nachkommen); Typisierung von 56 Mikrosatelliten in einem durchschnittlichen Abstand von 32 cM; Identifizierung von Chromosomenregionen mit Beziehung zur Heterosis für Wurfgröße. Chromosom 19 war mit Heterosis für Wurfgröße assoziiert. Daraufhin wurden zusätzliche Tiere mit extrem hohen und niedrigen Wurfgrößen für vier DNA-Marker auf Chromosom 19 typisiert und für die QTL-Kartierung benutzt. Es wurde ein QTL für Wurfgröße im Segment D19Mit28-D19Mit99 mit einem Maximum bei 15 cM gefunden ($p \leq 0.05$). Der QTL erklärt ca. 11% der phänotypischen Varianz in der F₂-Generation. Für den QTL zeigte ein Dominanzgrad von 4,09, daß Superdominanz die Heterosis für Wurfgröße erklären kann.

Schlüsselwörter: Heterosis, Fruchtbarkeit, Maus, DNA-Marker, QTL

Introduction

Heterotic effects are highly significant for animal breeding. As well known, trait values, which improve fitness of animals, are highly influenced by non-additive effects. For example, fertility is a trait with low heritability and indicates large heter-

otic effects (GÖTZ et al., 1991). Different types of crosses are suitable for the utilisation of heterosis whereas recurrent reciprocal selection (RRS) can improve non-additive effects. For RRS, the combination effects are measured from trait values after test matings of appropriate parents in the cross offspring. Test matings in farm animals increase the generation interval, cause financial losses and need high organization effort (LEE, 1997; BARBOSA-NETO et al., 1997). However, test crosses can be avoided, if homozygosity of DNA loci responsible for heterosis can be analysed in parents (LEUTHOLD, 1968). Superdominance, dominance, linkage and epistasis are assumed to be reasons for heterosis. Hardly another phenomenon of genetics is conflicted with numerous contradictory hypotheses as heterosis (POONI and TREHARNE, 1994; XIAO et al., 1996; STUBER, 1997). Molecular methods offer the possibility to verify the hypotheses of heterosis and to analyse the genetic basis of heterosis (TSAFTARIS, 1995). QTLs for traits with heterotic effects have been identified in plants (e.g. MITCHELL-OLDS, 1995; ARMSTEAD et al., 1997). Similar investigations are still missing in animals. In the work presented, DNA markers were used for QTL analysis of heterosis in litter size.

Material and Methods

4.1 Approach of heterosis analysis

The long term heterosis project is divided in three parts (Fig. 1). Litter size (number of total born animals, two litters) was used as selection criterium for fertility. Project part I is described in detail by BRUNSCH et al. (1997) and PHILIPP (1997). Reciprocal recurrent selection in part II of the project is directed to complementary homozygosity in the two lines.

In project part I, associations between a number of loci and litter size were analysed. Additional loci were considered for positions of chromosomes which had significant effects on litter size and genotyped in a larger number of animals (project part III). In the following contribution, first results are presented from project parts I and III.

4.2 Cross of the inbred lines C57BL/6J x Balb/cJ

The inbred lines C57BL/6J and Balb/cJ (Fig. 1) were mated reciprocally and subsequently the F₁ animals were intercrossed. Animals of inbred lines were purchased from Bomholtgard Breeding and Research Centre, Denmark. The F₂ generation consisted of 948 animals. Two extreme trait groups of litter size were selected from the 948 F₂ mice (18 animals with 13 offspring and 14 animals with 5 offspring). In part III additional animals with high or low litter sizes were included for QTL analysis of chromosome 19 (49 animals: 12 offspring; 49 animals: 7 offspring).

4.3 DNA analysis

In project part I two sets of DNA loci have been considered (PHILIPP, 1997): (1) 19 microsatellites which are localized close to loci with known associations to fertility, (2)

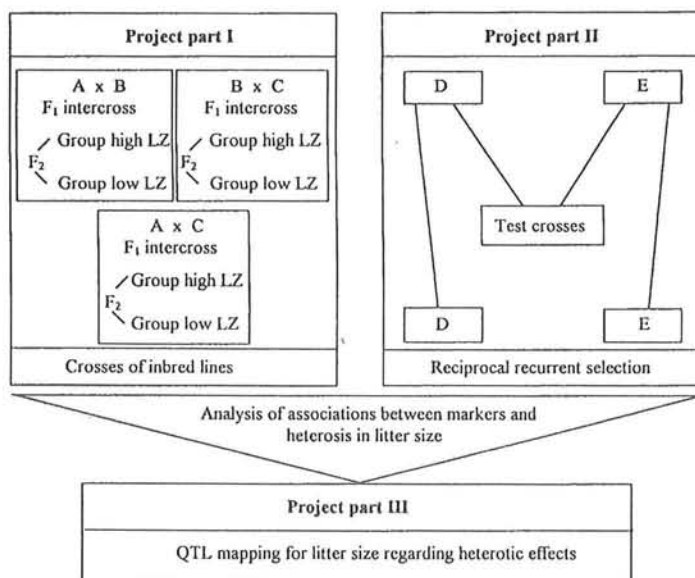


Fig. 1: Approach of heterosis analysis in mice (Ansatz zur Heterosisforschung bei Mäusen)

LZ: litter size

A, B, C: inbred lines C56BL/6J, Balb/cJ, NMRI/DKFZ

D, E: outbred lines, selected from F₂ animals of cross A x B

32 microsatellites evenly distributed throughout the genome. Additional markers were typed according to the results of project part I on chromosome 19 for which associations to heterosis were found. The microsatellites had an average distance of 14 cM on chromosome 19 (total length 55.7 cM) and the positions 3 cM (D19Mit93), 12 cM (D19Mit28), 20 cM (D19Mit98) and 41 cM (D19Mit99). Distances between loci are taken from Mouse Genome Database, August 1998.

The microsatellites were PCR amplified with non-labeled primers (1 x 180 s 94 °C; 25 x {15 s 94 °C, 120 s 56 °C, 120 s 72 °C}; 1 x 350 s 72 °C) and PCR products were separated in a 12 % polyacrylamide gel (Sequencing equipment SS1000, Fröbel, Wasserburg and electrophoretical equipment Blue Vertical 160C, Serva, Heidelberg). The staining of separated PCR products by silver nitrate followed (BASSAM et al., 1991).

4.4 Statistical analysis

The analysis of associations between genotypes of microsatellite loci and heterosis was performed using two models: superdominance (AA < Aa > aa) or dominance (AA = Aa > aa.). Associations between the degree of heterozygosity and litter size were investigated by Chi square tests according to PEARSON (SPSS, Version 6.1). In cases of observations per genotype smaller than five, the Exact Test has been used. The

CRI-MAP software of GREEN et al. (1990) was applied for linkage mapping. QTL mapping was performed using an interval mapping approach. Chromosome specific thresholds were estimated via permutation analysis. A Bonferroni correction was applied to obtain genome-wide thresholds.

5. Results

Heterotic effects. Litter sizes for inbred strains, F₁ and F₂ generations are shown in Figure 2. Heterotic effects were 45.3 % in the F₁ and 15.1 % in the F₂ generation.

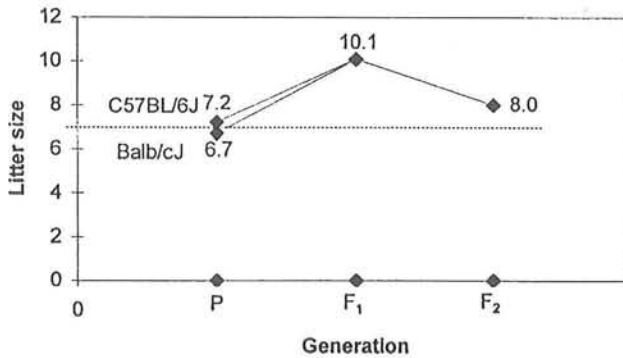


Fig. 2: Litter sizes in the inbred lines, F₁ and F₂ generation (Wurfgrößen der Inzuchtlinien, F₁- und F₂-Generation)

Associations between DNA markers on chromosome 19 and heterosis in litter size.

Table 1 gives the significant associations between the degree of heterozygosity of microsatellites and litter size in the extreme trait groups.

Table 1

Heterozygosity of microsatellites on chromosome 19 in the extreme trait groups of litter size (Heterozygotie der Mikrosatelliten auf Chromosom 19 der extremen Merkmalsgruppen für Wurfgröße)

Micro-satellite	Localisation ¹⁾ on chromosome 19 (cM)	Degree of heterozygosity (%)		p ²⁾
		High litter size (n=18)	Low litter size (n=14)	
D19Mit28	12	89	43	0.008
D19Mit61	8	89	43	0.008

¹⁾ Mouse Genome Database (MGD), August 1998

²⁾ Exact Test (SPSS, Version 6.1, Exact)

Linkage mapping of chromosome 19. The linkage map of chromosome 19 is shown in Figure 3.

QTL mapping for litter size. The plot of the test statistic (Figure 4) suggested a QTL for litter size on chromosome 19 in the interval D19Mit28 - D19Mit99 with the most likely position at 15 cM. Table 2 summarises the results of QTL analysis.

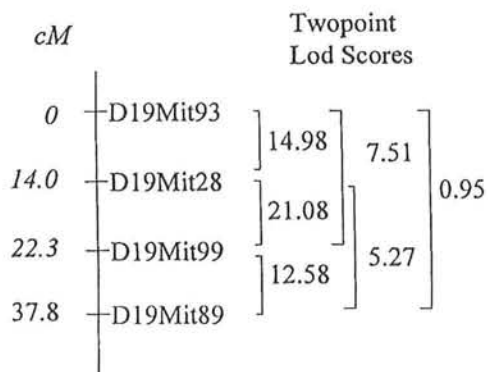


Fig. 3: The linkage map of chromosome 19 (Kopplungskarte von Chromosom 19)

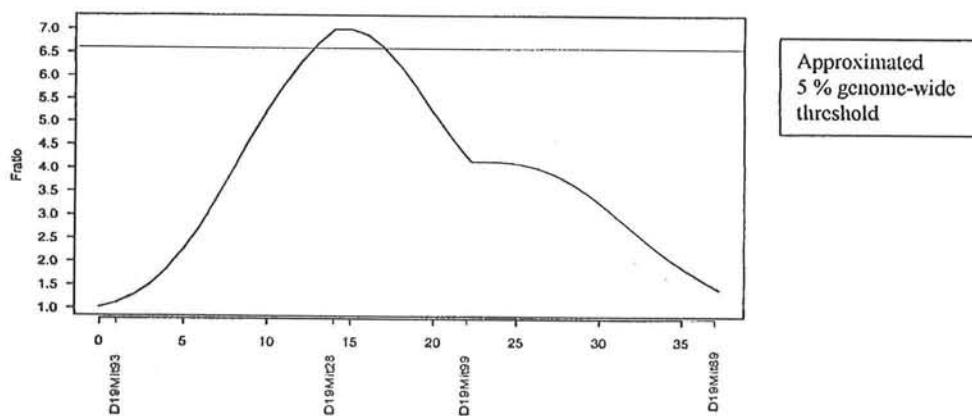


Fig. 4: Interval mapping on mouse chromosome 19 (Intervallkartierung von Chromosom 19 der Maus)

Table 2

Summary of QTL analysis on chromosome 19 (Zusammenfassung der QTL-Analyse auf Chromosom 19)

F ratio	7.01
F ₂ variance*)	10.92
Dominance effect (d)	3.00
±S.E.	±0.83
Additive effect (a)	0.733
±S.E.	±0.59
Degree of dominance (d/a)	4.09

*) Reduction on the residual variance in the F₂ generation by the QTL

6. Discussion

Mice are advantageous for heterosis research because of a short generation interval, small expenditure of housing, numerous inbred strains and abundant well characterised DNA markers. The litter size is a major parameter of fertility in mice as well as in all farm animals like pig. In agreement with literature data, high heterotic effect for litter size have been observed after cross of the inbred lines C57BL/6J and Balb/cJ (Fig. 2). So far four of 20 analysed chromosomes were associated with heterosis in litter size. Chromosome 19 was chosen for this study since the microsatellites D19Mit28 and D19Mit61 differed largely in their degrees of heterozygosity between the high and low trait groups and are in agreement with superdominance.

For linkage and QTL mapping of chromosome 19 additional animals and markers have been included. The calculated linkage map is in agreement with the MGD (1998). The results of QTL mapping strongly suggest the presence of a QTL on chromosome 19 at position 15 cM. The QTL explains about 11 % of the phenotypic variance in the F₂ generation. As a potential candidate gene of this chromosome region, the gene *Relaxin* with association to fertility is located at position 21 cM (MGD, August 1998). The high degree of dominance (4.09) shows that superdominance causes heterosis in litter size. Information on QTL obtained from mice can be transferred to farm animals. It seems possible to define DNA markers which are homologous between mice and other mammalian species and test them for heterosis in fertility. After identification of the responsible genes they can be used for breeding on non-additive gene effects.

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Buchbesprechung

Unsere Pferde - gesund durch Homöopathie

MICHAEL RAKOW

2. unveränderte Auflage, 224 Seiten, Sonntag Verlag, Stuttgart, 1998, ISBN 3-87758-170-6, 59,- DM, 431,- ÖS, 53,50 SFr

Naturheilkundliche Heilmethoden finden auch in der Tiermedizin zunehmend mehr Beachtung. Da nur wenig gute Fachliteratur verfügbar ist, die eine gute homöopathische Therapie beim Pferd ermöglicht, ist es ein besonderes Verdienst des Autors mit diesem Nachschlagewerk und Kompendium bessere Möglichkeiten homöopathischer Therapie aufzuzeigen. Dabei stützt er sich auf bewährte Standardwerke, vor allem aber auf seine langjährigen Erfahrungen in der homöopathischen Tiermedizin. Dieses Buch kann in der Aus- und Weiterbildung vor allem aber dem praktischen Tierarzt helfen die Symptome und homöopathischen Mittel zu finden, die zur Auffindung des heilenden Arzneimittels wichtig sind.

Nach einer Einführung in die Grundprinzipien homöopathischer Praxis, werden die wichtigsten Krankheitskomplexe, Erkrankungen und ihre homöopathische Behandlung beschrieben. Dabei wurde besonderer Wert auf die ausführliche Darstellung der Symptome gelegt, die bei einer bestimmten Erkrankung und einer bestimmten Symptomatik für die Arzneimittelwahl ausschlaggebend sind. Zum besseren Verständnis findet sich jeweils zu Beginn der Hauptkapitel, nach einer kurzen Erklärung des klinischen Sachverhaltes, eine Zusammenfassung der Fragen, die für einen bestimmten Krankheitskomplex relevant sind und es werden die für die Arzneimittelwahl wichtigen Symptome und vor allem Modalitäten aufgezeigt. Die Hauptabschnitte umfassen die Therapie der Atemwegserkrankung, der Krankheiten des Magen - Darmtraktes, des Bewegungsapparates, die Behandlung von Verletzungen, die Therapie bei Verhaltensstörungen und die homöopathische Geburtshilfe. Für die einzelnen Erkrankungen folgen nach Beschreibung der Ätiologie, die Symptomatologie und Therapie, gefolgt vom Spektrum möglicher Arzneimittel. Bei den jeweiligen Therapiekonzepten findet bei der Wahl eines homöopathischen Heilmittels die Gesamtheit der Symptome Berücksichtigung. Im Anhang finden sich Aussagen zum Arzneimittelrecht, zu homöopathischen Fachausdrücken sowie ein Arzneimittel-, Indikations- und Literaturverzeichnis.

Dieses Buch macht deutlich, daß man bei der homöopathischen Therapie von Pferden immer auf die genaue Beobachtung des Tieres durch den Tierhalter sowie den Tierarzt angewiesen ist. Durch die gute didaktische Aufbereitung kann dieses praxisrelevante Buch den notwendigen Beobachtungsaustausch zwischen Tierhalter und Tierarzt erheblich verbessern. Für den praktischen Tierarzt stellt das Buch eine Orientierungshilfe dar, die es ihm besser ermöglicht wichtige Angaben der Anamnese nicht zu übersehen, und es kann wesentlich zum Erfolg homöopathischer Heilmethoden beitragen. Es bereichert das Spektrum veterinärhomöopathischer Fachliteratur und ist einem breiten Leserkreis von Pferdehaltern und Tierärzten sehr zu empfehlen.

ERNST RITTER, Dummerstorf