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Hereditas 132: 55–62 (2000)

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Pinton, A., Ducos, A., Berland, H., Seguela, A., Brun-Baronnat, C., Darré, A., Darré, R., Schmitz, A. and Yerle, M., 2000. Chromosomal abnormalities in hypoprolific boars. —*Hereditas 132*: 55–62. Lund, Sweden. ISSN 0018-0661. Received July 5, 1999. Accepted January 24, 2000

Four new chromosomal rearrangements are reported in the domestic pig: 3 reciprocal translocations, rcp(4;12)(p13;q13) in a crossbred boar, rcp(1;7)(q17;q26) in a Large White purebred boar, rcp(1;6)(q17;q35) in a purebred synthetic paternal line boar, and a pericentric inversion inv(2)(p13q11) in a crossbred boar. The 1/7 reciprocal translocation and the pericentric inversion were detected in animals that had sired small litters. The effect of the 1/7 translocation was accurately determined: −4.5 piglets born per litter, i.e. −36 %. Both the 1/6 and 1/7 reciprocal translocations were of maternal origin. All the chromosomal rearrangements were highlighted using GTG and/or RBG banding techniques. Chromosome painting experiments were also carried out to confirm the proposed hypotheses for the three reciprocal translocations.

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A relatively large number of chromosomal abnormalities have been identified to date in the pig. The most frequent are reciprocal translocations (more than 70 distinct cases described: POPESCU 1989; LONG 1991; DUCOS et al. 1997a). These abnormalities are often responsible for a considerable decrease in prolificacy of the carrier animals or of the mates of carrier animals (DUCOS et al. 1996, 1997a). This degradation in reproductive performance can have serious economic repercussions for breeders (POPESCU and TIXIER 1984; BONNEAU et al. 1991). In order to minimize the cost of chromosomal abnormalities for the French porcine profession, various actions aimed to detect and eliminate such anomalies have been undertaken jointly by the professional breeding organizations and the French National Institute for Agricultural Research. An initial measure recently adopted by the majority of French selection organizations has consisted of a systematic chromosomal evaluation of the purebred boards used for breeding purposes in selection or multiplication herds via artificial insemination. This measure avoids using boars carrying reciprocal translocations (DUCOS et al. 1998a, 1998b) or other abnormalities (DUCOS et al. 1997b). In parallel, a statistical analysis of information relating to the reproductive performance of sows and boars in all the herds taking part in the National Programme of Technical Management of the Sow Herds (about 4000 herds in 1998) is being carried out quarterly. Approximately 50 % of the hypoprolific boars detected in this way were found to carry a chromosomal rearrangement (more than 10 reciprocal translocations have been identified in France thanks to this strategy—DUCOS et al. 1997a). Finally, an official rule has been adopted in 1999 by the National Commission of Genetic Improvement, stating that only those boars born in litters of sizes exceeding certain thresholds (which varies according to the breed) can from now on be approved for use in artificial insemination. For a boar born in a litter below the threshold size, approval will depend on chromosomal analysis to confirm the absence of anomaly. During the first half of 1999, 11 analyses were carried out in this context in our laboratory. They enabled us to highlight two new chromosomal rearrangements, namely a reciprocal translocation and a pericentric inversion, which are described in this paper (cases 1 and 4). The inversion reported here has also been identified in a hypoprolific boar. Two other new reciprocal translocations are also described, one in a boar that had sired small litters (case 2), the other in a young male controlled before reproduction (case 3). All these abnormalities were detected using traditional banding techniques. The interpretations proposed in the cases of reciprocal translocations (translocated chromosomes, localization of the breakpoints) were checked by chromosome painting.

MATERIALS AND METHODS

Animals

The first chromosomal rearrangement, rcp(4;12)-(p13;q13), subsequently described as case N° 1, was identified in a young crossbred boar (paternal lines) born in a litter of 6 piglets. The animal was 8 months old at the time of analysis. It was on standby, waiting for approval in an artificial insemination center (AIC).

The second anomaly, rcp(1;7)(q17;q26), case N° 2, was highlighted in a two year old hypoprolific purebred boar (Large White breed), that has been used for one year in a selection herd.

The third chromosomal rearrangement, rcp(1;6)-(q17;q35), case N° 3, was identified in a purebred boar (male composite line) controlled before reproduction at the selection level. It was eight months old at the time of analysis.

The fourth chromosomal abnormality, inv(2)-(p13q11), case N° 4, was identified in two related animals (kinship coefficient of 0.008). The first was an 18 month old crossbred boar detected as hypoprolific in a production herd. The second was a boar of the same genetic type, born in a litter of 6 piglets, on standby, waiting for approval in an AIC. He was 8 months old at the time of analysis.

Cases N° 2 and 4 were from the same breeding organization. Family analyses have been carried out in cases 2 and 3.

Cytogenetical analyses

Cytogenetical analyses were carried out on metaphasic chromosomes obtained from classical non-synchronized cultures of lymphocytes or fibroblasts. Two banding techniques were used: GTG-banding (SEABRIGHT 1974) and RBG-banding (DUTRILLAUX and COUTURIER 1981)—see DUCOS et al. 1998a, 1998b for more details.

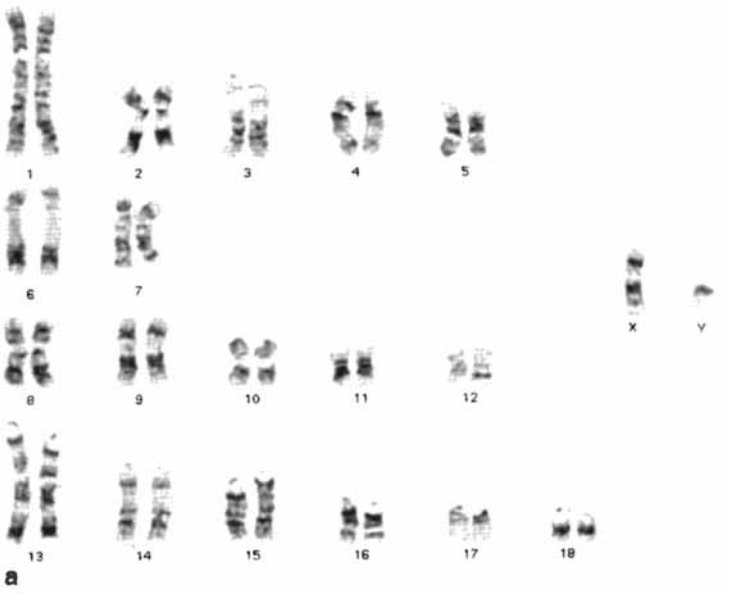
Dual-colour fluorescence in situ hybridizations (FISH) of whole chromosome probes (chromosome painting) were carried out to confirm the hypotheses put forward as a result of banding techniques in the cases of reciprocal translocations. The probes were produced by amplification of flow sorted chromosomes (see PINTON et al. 1998 and YERLE et al. 1993 for more details).

RESULTS

Cytogenetical analyses

The following descriptions (location of the breakpoints) need to be considered in relation to the standard G-banded karyotype (Committee for the Standardized Karyotype of the Domestic Pig 1988).

Case N° 1.—The examination of GTG-banded chromosomes underlined a translocated chromosome 4 on which the characteristic black (G-positive) and in p14 was no longer visible. In addition, the long arms of



a

Fig. 1a and b. a GTG-banding karyotype of a boar carrying the reciprocal translocation rcp(4;12)(p13;q13). The normal chromosomes are on the left, the rearranged chromosomes on the right. **b** Dual-color chromosome painting. Metaphase spread of a pig heterozygous carrier of rcp(4;12)(p13;q13).

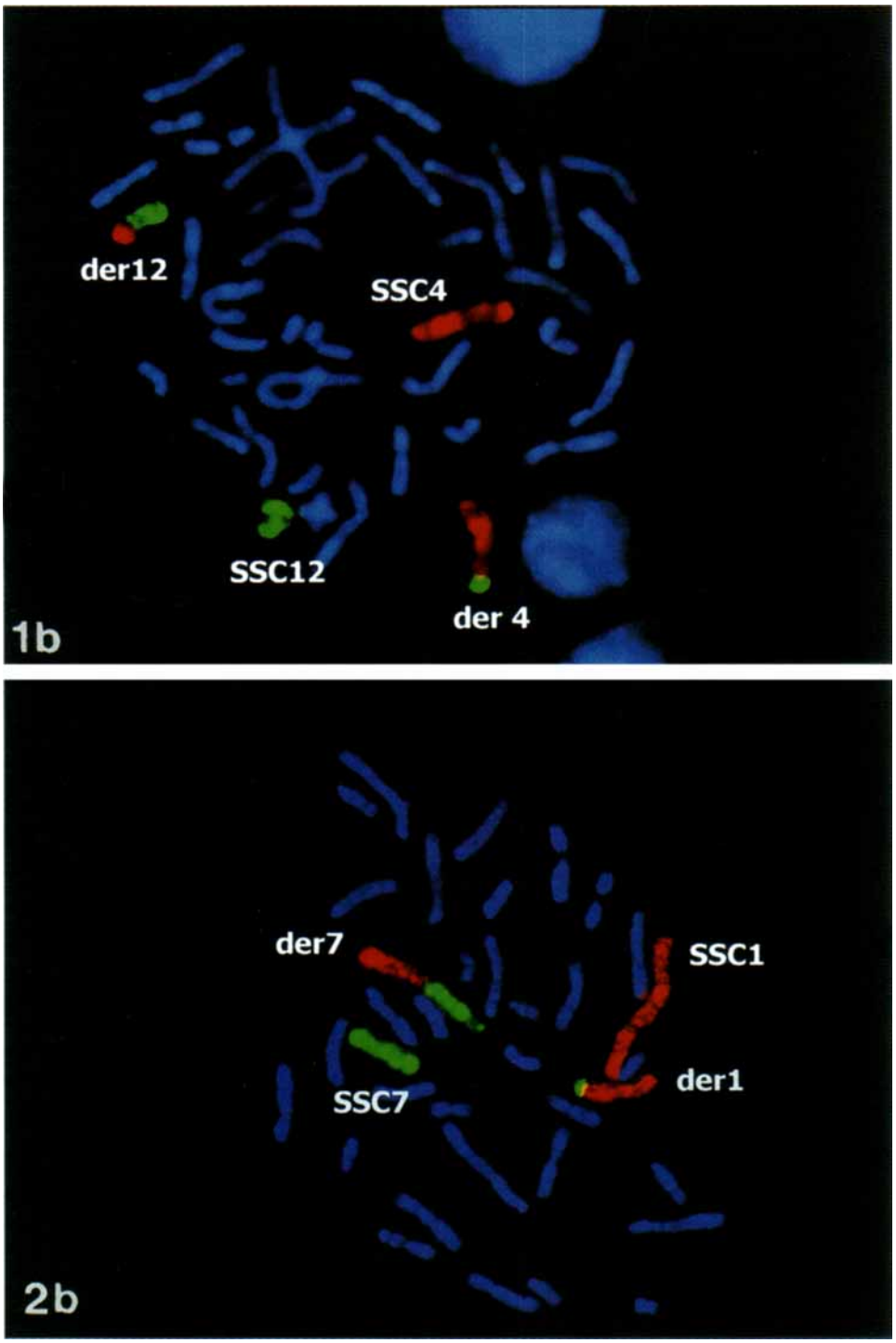


Fig. 1b, Fig. 2b

the abnormal (translocated) chromosome 12 presented a banding profile similar to that of the short arms of normal chromosome 4 (Fig. 1a). The breakpoints involved in this translocation seemed to be situated on bands 4p13 and 12q13. This assumption was confirmed by chromosome painting (Fig. 1b).

Case N° 2.—The examination of GTG-banded metaphases revealed one chromosome 7 in which the q-arms were abnormally long, as well as a chromosome 1 in which the q-arms were too short. The excess segment on the long arms of the rearranged chromosome 7 corresponded, without ambiguity, to

the final part (1q17→1qter) of the long arms of chromosome 1 (Fig. 2a). The presence of a fragment of chromosome 7 at the end of the translocated chromosome 1 was less obvious but could be confirmed by chromosome painting (Fig. 2b). The breakpoints involved in this translocation seemed to be situated on bands 1q17 and 7q26.

Case N° 3.—The observations permitting the identification of this rearrangement were very similar to those presented for case N° 2. The q-arms of one of the two chromosomes of pair 1 appeared shortened with an absence of fragment 1q1.7→1qter which was found at the end of the q-arms of a rearranged chromosome 6 (Fig. 3a). The breakpoint on chromo-

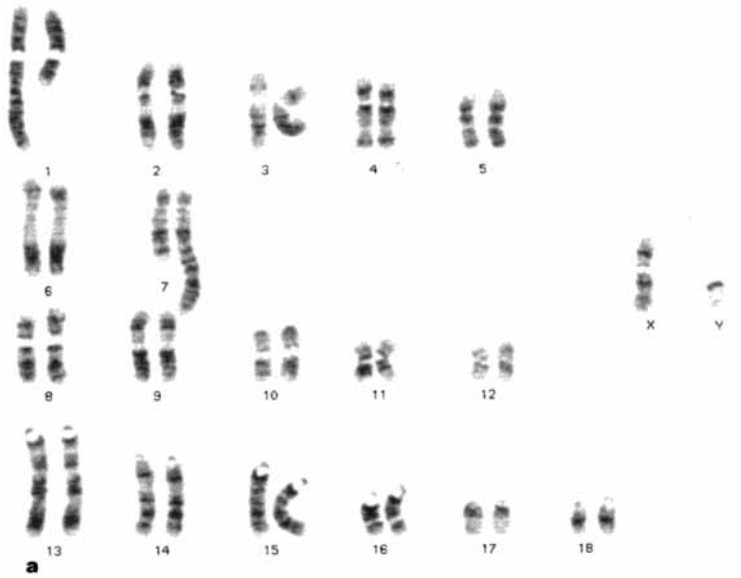


Fig. 2a and b. a GTG-banding karyotype of a boar carrying the reciprocal translocation rcp(1;7)(q17;q26). The normal chromosomes are on the left, the rearranged chromosomes on the right. **b** Dual-color chromosome painting. Metaphase spread of a pig heterozygous carrier of rcp(1;7)(q17;q26).

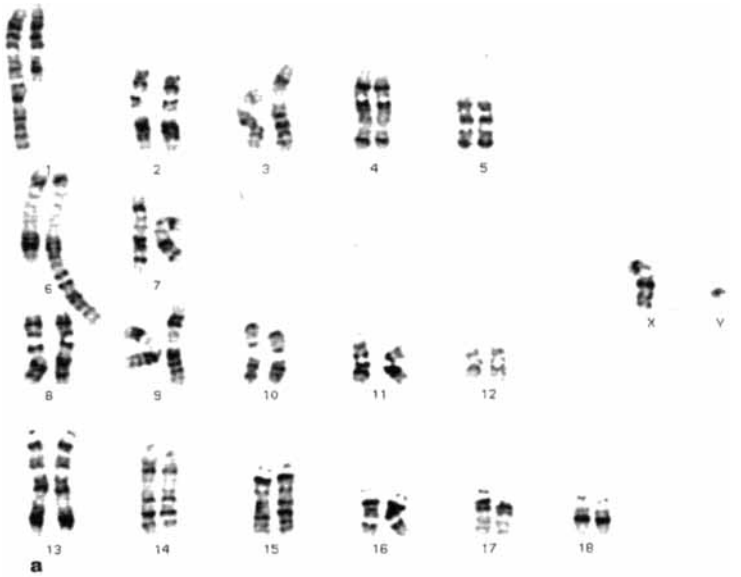
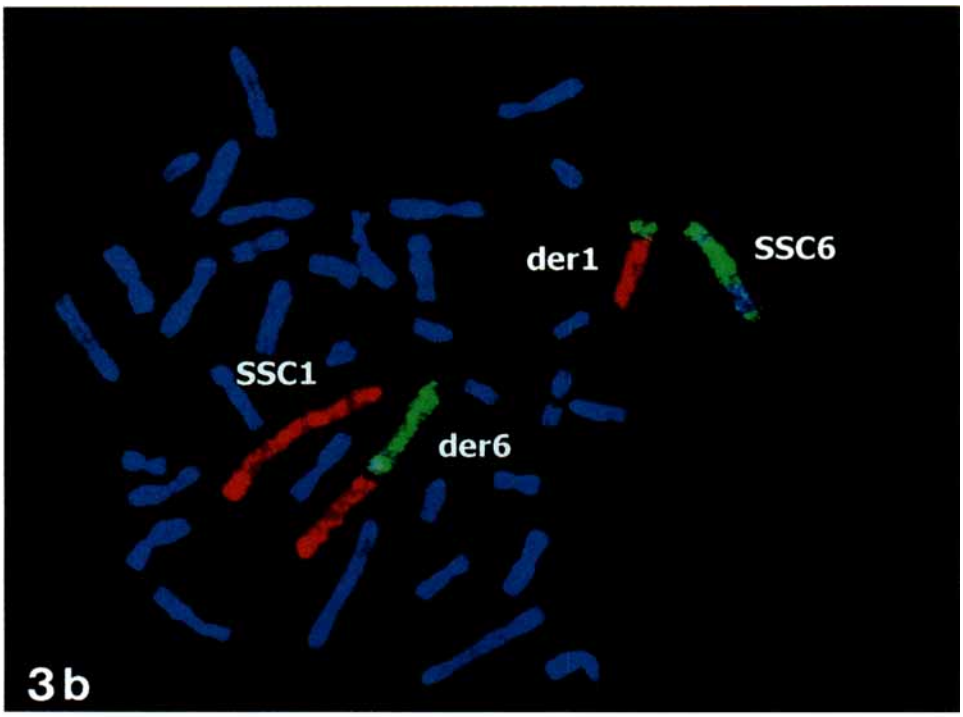


Fig. 3a and b. a GTG-banding karyotype of a boar carrying the reciprocal translocation rcp(1;6)(q17;q35). The normal chromosomes are on the left, the rearranged chromosomes on the right. **b** Dual-color chromosome painting. Metaphase spread of a pig heterozygous carrier of rcp(1;6)(q17;q35).



4b

Fig. 3b, Fig. 4b

some 1 seemed the same as in case N° 2 (1q17). The reciprocity of the translocation (presence of a fragment of chromosome 6 at the end of the q-arms of the translocated chromosome 1) was, once again, confirmed by chromosome painting (Fig. 3b). The chromosomal rearrangement could be described, according to the standard nomenclature, as 38,XY,rcp(1;6)(q17;q35).

Case N° 4.—In this case, the anomaly could be highlighted from the abnormal position of the centromere of one chromosome 2. The GTG appearance of all the other chromosomes of the karyotype was normal (Fig. 4a). Characterization of this anomaly by GTG-banding appeared to be particularly difficult. We therefore carried out RBG-banding which allowed better identification of the centromeres. The

RBG-banding profiles of the normal chromosome 2 and of the rearranged chromosome 2 seemed identical except for the centromeric region which was apparently inverted (Fig. 4b). This hypothesis seemed coherent with the observations made on GTG-banded chromosomes. The chromosomal rearrangement could be described, according to the standard nomenclature, as 38,XY,inv(2)(p13q11).

Zootechnical analysis

Two of the abnormalities described in this paper (cases N° 2 and 4) were identified in hypoprolific boars. Accurate information concerning the reproductive performance of the boar carrying the chromosomal rearrangement and of the contemporary boars were only available for case N° 2. The size of the litters sired by the translocated boar ranged from 3 to 16 (7.5 on average). As compared with the average litter size (12.5) sired by contemporary boars in the same herd, the 1/7 reciprocal translocation seemed to be responsible for a 36 % decrease in prolificacy (Fig. 5). The average size of the litters sired by the boar carrying the pericentric inversion (case N° 2) was considered to be abnormally low by the breeder but more accurate information that would allow us to estimate the effect of the anomaly was not provided by the breeding organization.

DISCUSSION

The chromosomal abnormalities presented in this paper have never been reported until now. Two of them concern chromosome 1. This chromosome, the largest in the pig karyotype, is involved in approximately one fourth of the reciprocal translocations reported in the literature (DUCOS et al. 1996, 1997a). Three distinct reciprocal translocations between chromosomes 1 and 6 have been described in the past: rcp(1;6)(1p − ;6q +) in the Large White breed (LOCKNISKAR et al. 1976), rcp(1;6)(p11;q11) in the Swedish Large White breed (YANG et al. 1992), and rcp(1;6)(q12;q22) in the Gasconne local breed (DUCOS et al. 1998a). As the breakpoints on the chromosomes and the breed of the carrier animals were different from those of the preceding cases, the translocation rcp(1;6)(q17;q35) presented in this paper seems to represent a new and distinct case. For the same reasons, the anomaly rcp(1;7)(q17;q26) differs from the translocation rcp(1;7)(q213;q24) identified and described by GUSTAVSSON et al. (1988) in the Swedish Large White breed. The reciprocal translocations involving chromosome 4 reported in the literature were, in chronological order, translocations 4/14 (POPESCU and LEGAULT 1979), 4/13 (MÄKINEN and REMES 1986), 4/15 (BLAISE et al. 1990), and 4/6 (DUCOS et al. 1998b). Once again, the reciprocal translocation 4/12 described in the present paper constitutes a new case. It was possible from the family analyses carried out on translocations 1/7 and 1/6 to demonstrate the maternal origin of both anomalies. The origin of the 4/12 translocation could not be investigated, for reasons beyond our control. The estimated effect of translocation 1/7 on reproductive performance (− 4.5 piglets born per litter on average, i.e. − 36 %) was relatively great and similar to the

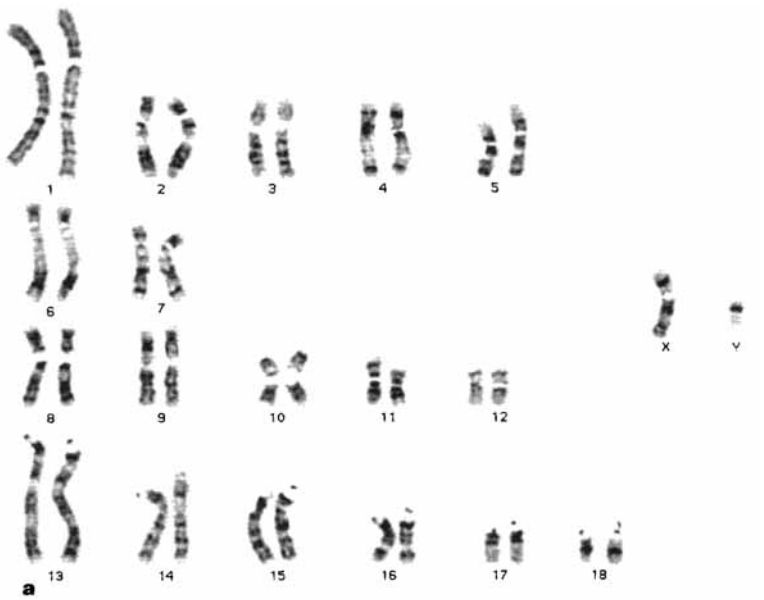


Fig. 4a and b. **a** GTG-banding karyotype of a boar carrying the pericentric inversion inv(2)(p12q11). The normal chromosomes are on the left, the rearranged chromosomes on the right. **b** RBG-banding chromosomes of pair 2: the normal chromosome is on the left, the inverted chromosome on the right.

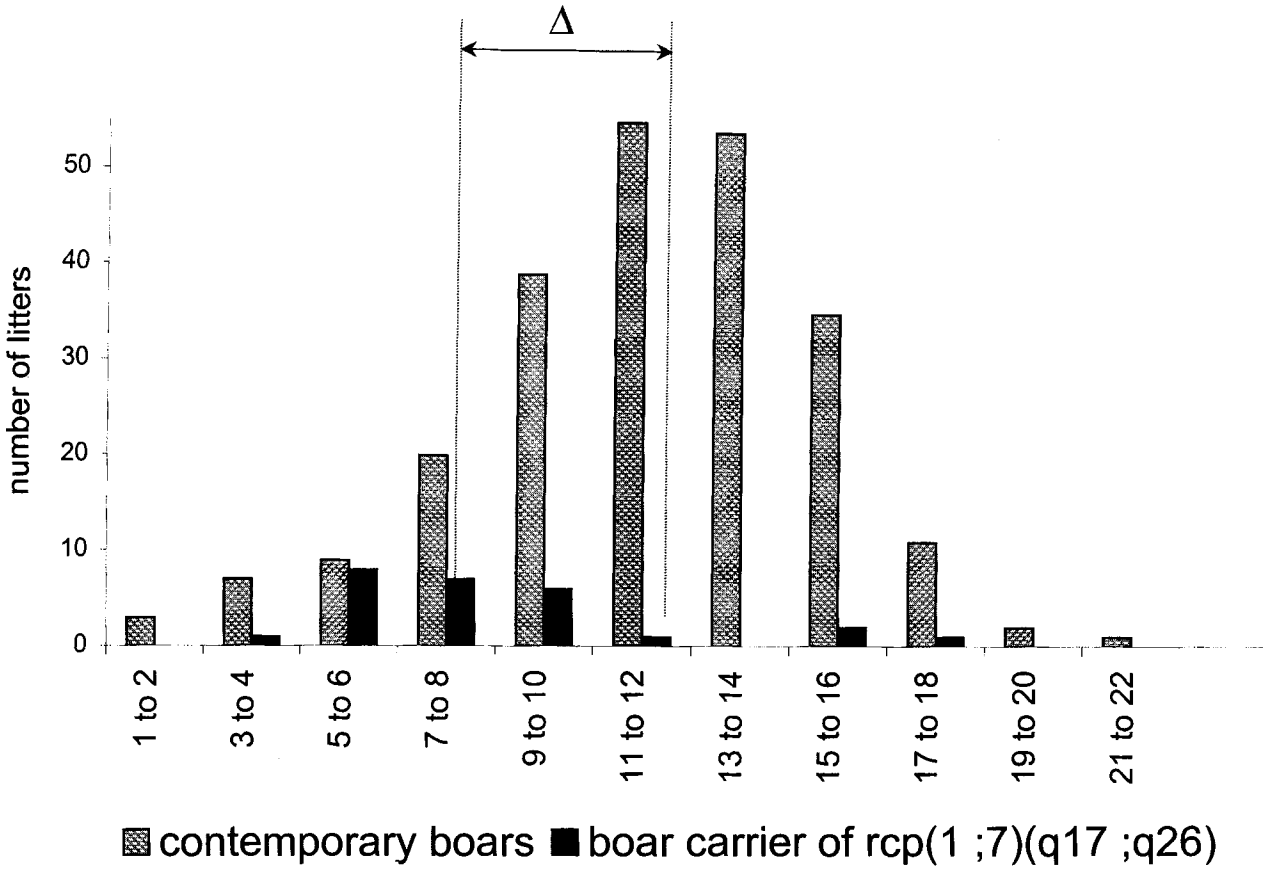


Fig. 5. Distribution of the litter sizes sired by the boar carrier of the rcp(1;7)(q17;q26) translocation, or by contemporary boars used at the same time in the same herd. △ represents the mean difference between the two categories (△ = 4.5 piglets).

average effect of − 40 % estimated from results in the literature (DUCOS et al. 1996). The effect of the 1/6 translocation could not be estimated accurately as the boar carrying the anomaly was killed before producing. The only available information was the reproductive performance of the boar's mother which also carried the translocation: two litters of 9 and 12 piglets, respectively, i.e. normal values in this paternal breed. No information was available to allow estimation of the effect of the 4/12 translocation on reproductive performance.

Although a large number of reciprocal translocations have been described in the pig, peri- or paracentric inversions are apparently much rarer in this species. To our knowledge, only 4 cases have already, and relatively recently, been reported: a paracentric inversion of chromosome 8 (SWITONSKI 1991), two pericentric inversions of chromosome 1 (DANIELAK-CZECH et al. 1996, MIYAKE et al. 1994) and a pericentric inversion of chromosome 4 (DUCOS et al. 1997b). These chromosomal abnormalities did not have significant effects on the animal's reproductive performance. The pericentric inversion of chromosome 2 presented in this paper thus constitutes the fifth case of inversion reported in the pig. The fact of having independently identified the same anomaly in two animals, one hypoprolific boar, the other born in a small litter (hypoprolific dam), would suggest a negative effect of this particular inversion on reproductive performance. This hypothesis could be investigated by analysing the gametes produced by the heterozygous carrier boar, using for example the recent and powerful sperm-FISH technique (JAAROLA et al. 1998).

The four chromosomal rearrangements described in this paper were identified using classical GTG or RBG banding techniques. The chromosome painting experiments allowed us to confirm the nature of the chromosomes involved in the reciprocal translocations and to locate the breakpoints more accurately. The reciprocity of the 1/6 and 1/7 translocations, difficult to prove with banding techniques, was also confirmed. FISH experiments were also carried out to confirm the hypothesis put forward, after banding analysis, in the case of the pericentric inversion of chromosome 2 (hybridization of a BAC probe con-

taining the WT1 gene, located on the 2p14-2q11 interval, i.e. within the inverted chromosome fragment—LAHIB-MANSAIS et al. 1997). Unfortunately, the results obtained until-now have not provided any additional information about this rearrangement.

The use of FISH techniques for the characterization of chromosomal abnormalities in domestic animal species is still far from frequent. To our knowledge, only four cases have been published (reviewed by PINTON et al. 1998). The main reason has been the difficulty of obtaining FISH probes in these species. The development and increasing availability of genomic libraries, collections of flow-sorted chromosomes, as well as the development and technological improvement of chromosome microdissection techniques (CHAUDHARY et al. 1998) should allow us to overcome these difficulties in the near future.

ACKNOWLEDGEMENTS

We wish to express our gratitude to the staffs of the insemination centres, of the selection organizations as well as the herd-stock breeders, for the blood-taking and all the arrangements concerning the animals.

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