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EVALUATION AND CONSERVATION OF EUROPEAN RABBIT (*ORYCTOLAGUS CUNICULUS*) GENETIC RESOURCES. FIRST RESULTS AND INFERENCES

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EVALUATION AND CONSERVATION OF EUROPEAN RABBIT (*ORYCTOLAGUS CUNICULUS*) GENETIC RESOURCES. FIRST RESULTS AND INFERENCES

BOLET G.[1], BRUN J.M.[1], MONNEROT M.[2] , ABENI F.[3], ARNAL C.[4], ARNOLD J.[5], BELL D.[6], BERGOGLIO G.[3], BESENFELDER U.[7], BOSZE S.[8], BOUCHER S.[5], CHANTELOUP N.[9], DUCOUROUBLE M.C.[10], DURAND-TARDIF M.[11], ESTEVES P.J.[12,13], FERRAND N.[12,13], GAUTIER A.[2], HAAS C.[7], HEWITT G.[6], JEHL N.[14], JOLY T.[15], KOEHL P.F.[14], LAUBE T.[10], LECHEVESTRIER S.[10], LOPEZ M.[4], MASOERO G.[3], MENIGOZ J.J.[5], PICCININ R.[16], QUENEY G.[2], SALEIL G.[1], SURRIDGE A.[6], van der LOO W.[17], VICENTE J.S.[18], VIUDES de CASTRO M.P.[19], VIRAG J.S.[20], ZIMMERMANN J.M.[5]

[1] Institut National de la Recherche Agronomique (INRA), SAGA, BP 27. F31326 Castanet-Tolosan Cedex, France. bolet@toulouse.inra.fr
[2] Centre National de la Recherche Scientifique (CNRS), CGM, Avenue de la Terrasse. F91198 Gif sur Yvette Cedex, France
[3] Istituto Sperimentale per la Zootecnia (ISZ), Via Pianezza, 115. 10151 Torino, Italy
[4] Universidad de Zaragoza (UNIZAR), Facultad de Veterinaria, Miguel Servet, 177. S50013 Zaragoza, Spain
[5] Fédération Française de Cuniculture (FFC), 28 rue du Rocher. F75008 Paris, France
[6] University of East Anglia (UEA), School of Biological Sciences, NR4 7TJ. Norwich, Great Britain
[7] Interuniversitäres Forschungsinstitut Für Agrarbiotechnologie (IFFA), Konrad Lorenz Strasse 20. A3430 Tulln, Austria
[8] Agricultural Biotechnology Center (ABC), Institute for Animal Science, PO Box 411, Gödöllö H2101, Hungary
[9] Institut National de la Recherche Agronomique, PAP, F37380 Nouzilly, France
[10] Centre d'Enseignement Zootechnique (CEZ), Ecole d'Aviculture, Parc du Château, F78120 Rambouillet, France
[11] Bureau des Ressources Génétiques (BRG), 16 rue Claude Bernard. F75231 Paris Cedex 05, France
[12], Faculdade de Ciências do Porto (FCUP), Departamento de Zoologia e Antropologia Praça Gomes Teixeira. P4050 Porto, Portugal
[13] Centro de Estudos de Ciencia Animal (CECA/UP), Campus Agrário de Vairão. P4480 Vila do Conde, Portugal
[14] Institut Technique de l'Aviculture (ITAVI), 28 rue du Rocher. F75008 Paris, France
[15] Institut Supérieur d'Agriculture Rhône Alpes, 31 Place Bellecour. F69288 Lyon Cedex 02, France
[16] Entente Européenne d'Aviculture et de Cuniculture, Rabbit Section, Wilhelminastraat 8. 6351 GN Bocholtz, The Netherlands
[17] Vrije Universiteit Brussel (VUB), Institute of Molecular Biology, Evolutionary Immunogenetics Research Group, 65 Paardestraat, Genesius-Rode. B1640, Belgium
[18] Universidad Politecnica (UPV), Ciencia Animal, Camino de Vera, 14, PO Box 22012. S46022 Valencia, Spain
[19] Universidad Miguel Hernandez, Division de Produccion Animal. S03312 Orihuela, Spain.
[20] Institute for Small Animal Research (KATKI), PO Box 417, Gödöllö. H2101, Hungary

ABSTRACT

In Europe, more than 60 breeds are registered by the national associations of rabbit breeders. However, these breeds are scarcely used for the commercial production of meat, which is based mainly on specialised strains. A European program (RESGEN CT95-060, regulation 1467/94, 1996-2000), co-ordinated by INRA, aimed at a more comprehensive description of these breeds and at evaluating ten of them at levels of both genetic diversity and zootechnical characteristics. Its preliminary results are presented in this article. From a questionnaire available in 6 languages and disseminated throughout Europe by the European association of rabbit breeders and the FAO national focal points, a data bank has been created including historical, morphological, demographic and basic zootechnical information. So far, more than 150 national breeds from 11 countries have been registered. This data bank should be available shortly on the EAAP and FAO (DAD-IS) web pages. A sample of 10 of these breeds was chosen for detailed examination, namely Argenté de Champagne, Belgian Hare, Chinchilla, English, Fauve de Bourgogne, Flemish Giant, French Lop, Himalayan, Thuringer, Vienna White,

plus the Hungarian Giant and Gigante de España breeds and a control strain from INRA. Characterisation of genetic diversity within and between breeds involved six laboratories. This was performed by using different sets of genetic markers: mitochondrial DNA, microsatellites (28 loci), protein polymorphism, analysed either at the level of genes products (25 different loci) or at the molecular level (κcaseins), immunoglobulins and MHC genes. Preliminary analysis indicates a strong genetic differentiation between breeds together with some structuring within the breeds. These domestic breeds show an allelic diversity dramatically lower than that of the wild populations taken as a whole; no significant reduction was observed when compared to the wild populations from which they originate, with the notable exception for the immunoglobulin loci. Zootechnical evaluation was performed on 5 experimental farms with a common control strain in three of these. Reproductive performances were generally low for all breeds except fertility in breeds of small body-size.. Preliminary results revealed a large diversity with respect to growth, carcass and meat quality traits and original features for some breeds, with potential economic interest. A cryobank was constituted, aiming at the conservation of well-characterised animals rather than endangered resources. Up to now, this cryobank has collected about 1500 frozen embryos and 360 semen straws from 8 breeds. Preliminary conclusions and possibilities for further developments are drawn.

RESUME

En Europe, plus de 60 races sont répertoriées par les associations nationales d'éleveurs de lapins de races. Cependant, ces races sont rarement utilisées pour la production commerciale de viande, qui utilise essentiellement des souches spécialisées. Un programme européen (RESGEN CT95-060, régulation 1467/94, 1996-2000), coordonné par l'INRA, s'est fixé comme objectif de fournir une description aussi complète que possible de ces races et d'évaluer la diversité génétique et les caractéristiques zootechniques d'un échantillon de dix d'entre elles. Les résultats préliminaires sont présentés dans cet article. A partir d'un questionnaire disponible en 6 langues et diffusé en Europe par l'Entente Européenne de Cuniculture et les points focaux de la FAO, une banque de données relationnelle a été créée; elle inclut des données historiques, démographiques, morphologiques et zootechniques. A ce jour, plus de 150 races nationales, provenant de 11 pays, ont été enregistrées. Cette banque de données sera prochainement disponible sur les sites Web de la FEZ et de la FAO (DAD-IS). Les 10 races choisies sont : Argenté de Champagne, Bélier Français, Blanc de Vienne, Chamois de Thuringe, Chinchilla, Fauve de Bourgogne, Géant des Flandres, Lièvre Belge, Papillon anglais et Russe, auxquelles s'ajoutent le Géant de Hongrie et le Gigante de España, ainsi que la souche INRA9077 comme témoin. Six laboratoires sont impliqués dans la caractérisation de la diversité génétique intra race et entre races, à partir de différent types de marqueurs génétiques : ADN mitochondrial, microsatellites (28 loci), polymorphisme de protéines, au niveau des produits des gènes (25 loci) ou moléculaire (caséines κ), gènes des immunoglobulines et du système majeur d'histocompatibilité. Les résultats préliminaires indiquent une importante différentiation entre les races ainsi qu'une certaine structuration génétique à l'intérieur des races. La diversité allélique dans l'ensemble de ces races est faible par rapport à celle de l'ensemble des populations sauvages, mais on n'observe pas de réduction significative de cette diversité par rapport aux populations sauvages dont les races domestiques sont issues, excepté pour les immunoglobulines. L'évaluation zootechnique a été réalisée dans 5 stations expérimentales, en présence de la souche témoin dans 3 d'entre elles. Les performances de reproduction sont faibles pour toutes les races, sauf la fertilité des races de petit format. En ce qui concerne les caractères de croissance, de carcasse et de qualité de la viande, les premiers résultats mettent en évidence une forte variabilité entre races; certaines races montrent des caractéristiques originales qui peuvent présenter un intérêt économique. Une cryobanque, dont l'objectif est plus de conserver des animaux bien caractérisés que des ressources génétiques menacées de disparition, est en cours de constitution ; à ce jour, elle rassemble environ 1500 embryons et 360 doses de semence de 8 races. A la suite des résultats, nous présentons les premiers enseignements et les prolongements possibles de ce programme.

INTRODUCTION

Domestic rabbit belongs to the species *Oryctolagus cuniculus* (European rabbit). It is the only domesticated mammal of Western European origin. Nowadays, wild and domestic populations still coexist. The history of domestication and formation of breeds has been described by Sandford (1992) and Arnold (1994). According to the latter, the first step after the antic period lied in hunting or keeping wild rabbits in warrens, from the Middle Ages to the 17th century. Domestication of rabbits took place during the 18th and mainly the 19th

centuries. The most important step of creation of breeds occurred during the first half of the 20th century. Meanwhile, societies of breeders were created to maintain and improve these breeds (Boucher, 1995). Nowadays, national associations of rabbit breeders exist in many European countries; they are in charge of the making and updating of the standard book of rabbit breeds, the exhibitions and competitions. They are particularly active in Germany or Switzerland, where they include thousands of breeders, but also in France, Italy, Belgium and the United Kingdom. Most of them are federated to the European Association of Poultry and Rabbit Breeders. This association issues a standard book, which describes more than sixty breeds bred in at least three different countries belonging to the association.

This description hides a deep ditch between pure breeding organisation and rabbit meat production. World rabbit meat production is running to 1.5 million tons/year and Europe is the main producer (around 65 percent) (Colin and Lebas, 1996). In Europe (mainly Italy, France, Spain,..), most of this production is realised using specialised herds whose size is gradually increasing and middle-sized herds within mixed farming. In both cases, the production is mainly based on crossbred meat rabbits obtained from very few commercial strains disseminated through pyramidal systems. These commercial strains were created from some middle-sized breeds (mainly New Zealand White, Californian) and few heavy breeds for paternal lines. In a few cases, they were crossbred with other breeds or local populations to produce synthetic lines.

So, the role of pure breeding in meat production is dramatically weak. However, these breeds present a wide range of characteristics and constitute a unique reserve of genetic variability: the diversity of their adult size, growth, conformation, coat colour, fur type,… is well known; their potential diversity concerning zootechnical performances and genetic polymorphisms has hardly been studied. Consequently, there are at least two main reasons to characterise and conserve rabbit breeds:

- Rabbit meat production is in the process of diversification, concerning the type of animals, the way of rearing, but also including non-alimentary uses; it could take advantage of new knowledge concerning breed diversity.
- Given the advances in molecular biology tools and knowledge, specific genetic characteristics of these breeds may shortly be evidenced and utilised.

This was the reason why a European program of inventory, characterisation, evaluation and conservation of European rabbit genetic resources was proposed and approved in 1996. The objectives and the experimental design have been described by Bolet *et al* (1999). We are now able to present the first results and to draw some general inferences from them with regard to these objectives.

INVENTORY OF BREEDS AND CREATION OF A DATA BANK

Most national associations of fancy rabbit breeders have done a primary phenotypic characterisation of the breeds they manage (national breed standards, e.g. Anonymous, 2000). The European Association, mentioned above, has published a European standard of breeds (Anonymous, 1995), which tries to synthesise the national standards. It gives a detailed description of the shape, conformation, size, fur, coloration,...of a total of 66 breeds. But the rabbit is missing from the FAO /DAD-IS (Hammond and Leitch, 1997) and the European Association for Animal Production (EAAP) (Hannover) data banks on domestic animal breeds, which give information on the census of animals, average zootechnical traits,... so that we have no other centralised information than their description.

To obtain such information, a questionnaire has been assembled in six languages (French, English, Spanish, Italian, German and Russian). It was elaborated and tested by the

"Federation Française de Cuniculture" (French national association of rabbit breeders); thereafter, it was sent to the other associations through the "European Association" and to the FAO European focal points through the "Bureau des Ressources Génétiques" (BRG, French FAO focal point). The questionnaire and the standards have been used to develop a database. Each breed association was asked to collect the necessary inquiries from breeders and bibliography on the breed in its country. If no breed association exists, breeders have been reached individually. The objective was to nearly completely collect as objectively and as homogeneously as possible data about rabbit breeds and strains raised in Europe. These data complete the historic and phenotypic description assessed by demographic and zootechnical information. So, the questionnaire makes mention of the description, utilisation, breeding management, zootechnical performances and breed conservation in each country. So far, more than 150 national breeds have been registered from eleven countries (Belgium, Czech republic, Denmark, France, Hungary, Italy, Luxembourg, Malta, Poland, Spain, Switzerland). These include standardised breeds but also commercial strains, in order to realise a description, as complete as possible, of rabbit genetic resources available nowadays in Europe. The questionnaire is available on the WRSA web page (www.rabbit-science.com)
The construction of the computerised data bank is now running (Ducourouble *et al*, 1999). It is being constructed with the ACCESS program (Microsoft). This relational database is constituted of tables (which are the support to stock information); these tables are constituted by fields containing the data. The tables are linked to each others ensuring a coherent, exhaustive and non-redundant stocking of information. The database may be consulted in three different ways by country, breed classification (giant, medium, small, dwarf, fur breed or commercial strain) or name of a breed in its country of origin. The part of this data bank concerning French breeds and strains is available on the BRG web page (www.brg.prd.fr). The whole data bank should shortly be available on the EAAP and FAO/DAD-IS web pages.

CHOICE AND SAMPLING OF BREEDS

1. Choice of breeds

Within this program, only a part of the breeds which were inventoried, approximately ten, could be evaluated. These breeds were chosen according to three criteria:
- those presumed to be among the oldest ones, or to originate from crossbreeding of the oldest ones,
- those present in various European countries,
- those having a potential zootechnical interest.

The 10 breeds chosen were:
- two heavy breeds: Flemish Giant (FG) and French Lop (FL),
- five medium-sized breeds: Belgian Hare (BH), Vienna White (VW), Argenté de Champagne (AC), Thuringer (TH), Fauve de Bourgogne (FB),
- three small-sized breeds : Chinchilla (CH), Himalayan (HI), English (EN).

These ten breeds were compared to the INRA9077 strain, used as a control (C77). It was created in 1976 from the New Zealand White breed and has been maintained without selection since then and used as a control strain for selection experiments (Rochambeau, 1998).
Additionally, we included the description of the genetic polymorphism of the Gigante de España (GE), a breed in risk of extinction which is conserved at the Veterinary school of Zaragoza (Sierra and Lopez, 1990; Medjoub *et al*, 2000). We also added the genetic and

zootechnical evaluation of the Hungarian Giant (HG) breed, in the frame of a specific collaboration with Hungarian research institutes.

2. Sampling of animals

The objective was to build up a sample of the above-mentioned breeds by buying around 30 females and 20 males per breed, as unrelated as possible. Table 1 shows that it was almost never possible to reach this objective. However, the animals bought originated from 15-20 different sires in most cases. Except for specific breeds (Hungarian Giant, Gigante de España, and the Control strain), each breed was sampled in at least two countries. These animals were used to study genetic polymorphism and to produce progeny for the zootechnical evaluation in comparison with the control strain.

Table 1. Evaluated breeds (grouped by body size) and characteristics of the sampled animals

Breed		Body size [1]	Country[2]	Founders	Breeders[2]	Sires[3]	Dams[3]
Control strain	C77	M	FR	25	1	18	24
English (butterfly)	EN	S	FR, SW	38	13	23	35
Himalayan	HI	S	FR, SW	28	11	13	13
Chinchilla	CH	S	FR, SW	33	12	9	12
Fauve de Bourgogne	FB	M	FR, IT	60	25	41	42
Thuringer	TH	M	FR, IT, SW	51	15	20	22
Vienna White	VW	M	FR, SW	33	14	18	19
Belgian Hare	BH	M	FR, IT	29	11	17	21
Argenté de Champagne	AC	M	FR, SW	29	9	12	16
French Lop	FL	L	FR, HU	44	6	8	14
Flemish Giant	FG	L	FR, IT	9	3	6	9
Hungarian Giant	HG	L	HU	29	5	13	17
Gigante de España	GE	L	SP	25	1	22	19

[1] S=small, M=medium, L=large body size
[2] FR=France, HU=Hungary, IT=Italy, SP=Spain, SW=Switzerland
[3] Number of animals bought, used to study genetic polymorphism and to produce offspring for zootechnical evaluation
[4] Number of parents (sires and dams) of the founder animals.

CHARACTERISATION OF THE GENETIC DIVERSITY

Some laboratories have already been working together on the characterisation of genetic polymorphism in several wild and domestic populations from Portugal, Spain and France (Ferrand, 1995; Monnerot *et al*, 1994, 1996; van der Loo *et al*, 1991, 1999 ; Vachot, 1996; Queney *et al*, submitted). They evidenced that the genetic diversity found in domestic rabbits was lower than that of wild populations as a whole ; however, it was not dramatically lower than that of wild populations of recently colonised areas (mainly France in these studies), from which they originate. But the number of animals sampled per domestic breed did not allow the genetic diversity between breeds to be studied. In this program, different sets of genetic markers were used to characterise the genetic diversity of the 13 breeds under control:
- mitochondrial DNA (mtDNA), which is almost exclusively maternally inherited, allows to assign animals to a precise maternal lineage,

- microsatellites, which are becoming the markers of choice for molecular population genetics, represent an important source of polymorphic genetic markers, supposed to be neutral, which are easily and rapidly scored,
- genes in relation with the immune system of animals : immunoglobulins and MHC (Major Histocompatibility Complex) genes,
- protein polymorphism, analysed either at the level of genes products (25 different loci) or at the molecular level (κcaseins).

All these markers were analysed from blood extracted from the founder animals, sent to the different laboratories and used as described by Bolet *et al* (1999).

For the moment, the whole analysis of blood samples and data has not yet been realised. Here, we present only preliminary results considered separately for each laboratory and set of markers. In the final step, these data will be analysed together, in order to draw a complete description of the patterns of genetic diversity within and between breeds. In this first step, except for mtDNA, the variability within breed and the differentiation between breeds were evaluated using :

- the number of samples per breed (N),
- the average number of alleles per gene or haplotype (A),
- the expected (He) and observed (Ho) heterozygosity rate,
- F-Statistics (F_{IS} and F_{ST}, varying from 0 to 1) defined by Wright (1951); they were calculated according to Weir and Cockerham (1984) using Genetix 4.0 and FSTAT 2.8 (for most laboratories) : intrapopulation structure was investigated using the F_{IS} parameter (observed individual heterozygosity compared to the theoretical one within breed; 0 means that the samples are from a panmictic population at Hardy-Weinberg equilibrium). Genetic differentiation between populations was estimated from the F_{ST} parameter (breeds heterozygosity compared to the overall heterozygosity; 0 means no differentiation between breeds).

1. Mitochondrial DNA (CNRS, France)

1.1 RFLP analysis

This analysis benefits from previous studies which evidenced that only two RFLP types were present in domestic rabbits (B1 and B3-4, Vachot, 1996; Queney *et al*, submitted).

Table 2 presents data for almost all the individuals involved in the project. According to the maternal inheritance of mtDNA, kinship was considered and data were also expressed taking the 332 different dams (maternal lineages) thus sampled into account.

Two regions of mtDNA were assessed : part of the cytochrome b gene for distantly related animals and part of the main non-coding domain for closely related animals. Various pairs of primers specific for these two regions define mtDNA portions of different lengths and phylogenetic signals. Once amplified by PCR, these portions are analysed either by sequencing or by RFLP (Hardy *et al*, 1995 ; Monnerot *et al*, 1996 ; Mougel, 1997).

Four groups, except the Flemish Giant breed because of the low number of animals characterised, were defined according to the percentage of B1 profiles (and the classification held at both individual and maternal lineage levels): 1) two breeds (AC and TH) and the control strain were pure B1; 2) a set of 2 breeds (FB and EN) exhibited a very high level of B1 (94 and 96 %); 3) the next 7 breeds formed a heterogeneous group from nearly 50 % up to more than 80 % B1; 4) the last breed (Gigante de España) exhibited only 11% of B1 thus being the only one for which the B3-4 profile was largely the majority. The overall mean of B1 reached almost 80%. The value given for the French Lop breed has to be considered with caution because of great disparity of the origin of the animals.

For each breed, the relatively high number of both individuals and maternal lineages probably accounted for the absence of bias when they were considered separately. In addition, the differences in B1 percentages from one breed to another did not appear correlated to differences in the number of breeders.

Table 2. Characterization of breeds through mitochondrial DNA RFLP typing

	Individuals			Maternal lineages		
Breeds**	Number samples	% B1* profile	% B3-4* profile	Number samples	% B1* profile	% B3-4* profile
Argenté de Champagne	29	100	0	16	100	0
Thuringer	51	100	0	34	100	0
Fauve de Bourgogne	60	97	3	50	96	4
English	38	95	5	35	94	6
Chinchilla	33	85	15	27	81	19
Vienna White	33	76	24	24	75	25
Himalayan	28	64	36	24	71	29
Belgian hare	29	59	41	22	55	45
Flemish Giant	9	56	44	9	56	44
Hungarian Giant	29	52	48	19	58	42
French Lop	44	45	55	29	48	52
Gigante de España	25	8	92	19	11	89
Control strain	25	100	0	24	100	0
Total	433	76	24	332	77	23

* According to Mougel, 1997

** Breeds are listed according to the decreasing percentages of B1 profile within maternal lineages.

1.2 Sequence analysis

Sequencing (500 bp) in the non-coding domain of mitochondrial DNA allowed to appraise the variability within each RFLP type. Seventeen different sequences were recognised from the analysis of 330 maternal lineages (only two were missing): 8 within the RFLP B1 type and 9 within RFLP B3-4 type. Eighty-six percent of the maternal lineages fell into two quite divergent (2.2%) sequence types: 001 and 008 which are, so far, the most frequent ones in French wild populations (Mougel, 1997; unpublished results) and are representative of RFLP types B1 and B3-4 respectively. Almost all of the rare sequence types (from 0.2 to 0.8 % nucleotide difference when compared to the related sequences 001 or 008) were only found in one breed, in one place and in animals coming from a relatively low number of breeders (9 out of the 15 rare types characterised rabbits from one breeder only).

The samples studied probably yielded an assessment which was very close to the overall mitochondrial diversity of any breed (except the French Lop). Most of the rare sequence types might be relevant of mutations that have arisen since the original selection of related breeds. Mitochondrial diversity of domestic breeds, sampled here and considered as a whole, is very similar to that of wild populations from France if also considered globally (Mougel, 1997; unpublished results).

2. Neutral diversity : Microsatellites

Microsatellites commonly occur in eukaryotic genomes and consist of stretches of mono- to penta-nucleotide motifs which are randomly repeated, dispersed throughout the whole

genome and usually flanked by unique sequences. Differentiation of very closely related populations is possible based on allele frequencies as well as diagnostic alleles. A set of 20 to 30 microsatellite loci already identified and available were used (Rico *et al*, 1994; Mougel *et al*, 1997; Surridge *et al*, 1997; Van Haeringen *et al*, 1997). Some of them have already been shown to be useful in characterising wild and domesticated populations (Vachot, 1996; Mougel, 1997; Surridge, 1997). In this preliminary step, data from the two laboratories dealing with microsatellites were analysed separately. They will be analysed together with the other genetic polymorphisms later.

2.1 CNRS Microsatellites (France)

Nine polymorphic microsatellites were used (Mougel, 1997). The mean number of alleles per locus varied from 3.0 to 4.2 and the expected heterozygosity varied from 0.443 to 0.583. The genetic diversity was thus relatively low but no significant difference appeared from one breed to the other. For most breed, the observed heterozygosity was lower than the expected heterozygosity.

Table 3. Genetic variability parameters for 9 microsatellites

Breed	N	A	H_e	H_o
Argenté de Champagne	29	3.8	0.508	0.468
Belgian Hare	29	3.0	0.443	0.393
Chinchilla	33	3.7	0.556	0.442
English	38	3.8	0.502	0.426
Fauve de Bourgogne	60	4.1	0.518	0.407
Flemish Giant	9	3.2	0.508	0.383
French Lop	44	4.1	0.556	0.568
Himalayan	28	3.8	0.524	0.373
Hungarian Giant	29	4.1	0.548	0.559
Gigante de España	25	3.2	0.480	0.497
Thuringer	51	3.3	0.537	0.523
Vienna White	33	4.2	0.529	0.444
Control strain	25	3.7	0.583	0.502
Mean values	33	3.7	0.522	0.460

N : sample size
A : mean number of alleles per locus
H_e : non-biased expected heterozygosity (Nei 1987)
H_o : observed heterozygosity

Estimators of F-Statistics , F_{ST} and F_{IS} were respectively 0.173 with 95% confidence interval [0.152 ; 0.201] and 0.114 with [0.058 ; 0.198]. A value of F_{ST} significantly higher than zero indicates a relatively strong genetic differentiation between breeds. The markers used here were tested for Mendelian inheritance (Mougel *et al* 1997) and followed Hardy-Weinberg expectations in wild populations (Mougel, 1997). So, the deficit in heterozygotes revealed by a high value of F_{IS} (significantly higher than zero) probably indicates a structuring within the breeds observed in the present study, which probably originated from the way sampling was performed at various localities and with different breeders.
The genetic diversity, appraised through the characterisation of these 9 microsatellite loci, appeared relatively low in comparison with wild populations (Mougel, 1997). Although

patterns of diversity were very similar, high value of F_{IS} relative to F_{ST} suggested an apportionment of genetic diversity among breeders within each breed.

2.2 UEA Microsatellites (Great Britain)

Table 4. Parameters of genetic variability for 19 microsatellites

Breed	N	A	He	Ho
Argenté de Champagne	29	3.3	0.302	0.274
Belgian Hare	29	2.7	0.205	0.171
Chinchilla	33	3.6	0.299	0.195
English	38	3.7	0.297	0.252
Fauve de Bourgogne	59	4.1	0.308	0.229
Flemish Giant	9	2.0	0.151	0.146
French Lop	39	3.4	0.265	0.221
Himalayan	28	4.1	0.348	0.256
Hungarian Giant	22	2.6	0.173	0.158
Thuringer	54	4.4	0.234	0.177
Vienna White	33	4.2	0.326	0.266
Control Strain	25	3.0	0.233	0.202

(N, A, He, Ho : see table 3)

Global $F_{IS} = 0.218 \pm 0.024$
Global $F_{ST} = 0.169 \pm 0.010$

3. Proteins polymorphism (FCUP, Portugal)

Genetic characterisation of domestic rabbit breeds was made by means of conventional electrophoresis and isoelectric focusing techniques for a total of approximately 25 protein loci detected in the blood (Ferrand, 1995). The average sample size for seven rabbit breeds analysed up to now as well as measures of genetic variability (heterozygosity and mean number of alleles per locus) were calculated according to standard procedures (Table 5).
The results showed that these breeds are not very different when classical parameters of genetic variability are compared. A fine scale analysis showed that rabbit breeds share most of the alleles detected. A notable exception, however, is the presence of allele HBA*6 in English, a new variant that was never observed in any other domestic or wild population. On the whole, a slight trend for fixation was detected in the Belgian Hare, Chinchilla and Himalayan breeds (relatively low values of A and He; see Table 5).
The global F_{IS} value (0.083) may be considered high and certainly reflects the relatedness between animals within breeds. This is especially true in this case because Mendelian inheritance of the polymorphic markers used in this study has been extensively demonstrated by different authors through breeding experiments as well as the conformance to Hardy-Weinberg proportions. The global value of F_{ST} (0.312) was unexpectedly high, due to important differences in gene frequencies between the breed samples analysed here. This result must, however, be interpreted with caution because at this moment we cannot discard the possibility of a sampling bias.
A comparison of allelic diversity values with those described for wild rabbits originating either from France or from the Iberian Peninsula showed a marked difference between all domestic breeds and wild rabbits from Portugal or Spain. This was especially visible for 'A' values, that were similar for French wild rabbits and domestic breeds (1.7 and 1.5,

respectively), but much higher in Iberian wild rabbits (2.6). These results are in agreement with the hypothesis that the genetic diversity observed in present day domestic breeds is only a subset of that observed when the whole species is considered (Ferrand, 1991).

Table 5. Parameters of genetic variability for 25 protein loci

Breed	N	A	He	Ho
Argenté de Champagne	21	1,52	0,170	0,197
Belgian Hare	20	1,44	0,114	0,100
Chinchilla	11	1,32	0,107	0,109
English	24	1,56	0,162	0,108
Fauve de Bourgogne	30	1,56	0,158	0,156
Himalayan	11	1,36	0,112	0,108
Vienna White	14	1,56	0,143	0,120
Mean values	18,7	1,47	0,138	0,128

(N, A, He, Ho : see table 3)

4. Casein polymorphism (ABC, Hungary)

Table 6. Parameters of genetic variability for the κcasein gene

Breed	N	A	He	Ho
Argenté de Champagne	29	2	0.313	0.379
Belgian Hare	27	2	0.453	0.370
Chinchilla	31	1	0.000	0.000
English	37	2	0.151	0.108
Fauve de Bourgogne	60	2	0.182	0.100
Flemish Giant	9	2	0.471	0.222
French Lop	37	2	0.424	0.324
Hungarian Giant	18	2	0.356	0.333
Himalayan	25	2	0.150	0.160
Thuringer	53	2	0.019	0.019
Gigante de España	25	2	0.503	0.400
Vienna White	33	2	0.116	0.121
Control strain C77	23	2	0.415	0.391

(N, A, He, Ho : see table 3)

κ-casein genotypes were determined by the size difference in the first intron. PCR was performed on genomic DNA (Bösze *et al*, 2000). Genomic DNA was isolated from blood samples by the standard protocol. The κ-casein genotype distribution in the thirteen examined breeds underlined our earlier observation obtained in two populations of NZW rabbits (Hiripi *et al*, 1998). The frequency of the A allele was found to be higher in 12 breeds. In the Chinchilla breed, the presence of the B allele was not detected. The higher F_{IS} value (0.16) when compared to F_{ST} (0.10) could be related to the fact that only one locus was concerned and bias can easily occur as in the Chinchilla. Preliminary data obtained from the analysis of wild populations of European rabbits from its original range showed the presence of both κ-casein alleles in all of them.

αs1-casein and αs2-casein protein polymorphisms were determined by a modified version of isoelectric focusing of milk samples (Baranyi *et al* 1995). Eighty-eight milk samples from 11

breeds have been collected and evaluated so far; however there is not yet enough data for the statistical analysis.

5. Immune system genes

5.1 Haplotypic diversity of the Major Histocompatibility Complex (INRA, France)

In all species, MHC is the best 'identity card' of individuals, possibly playing a role in individual recognition. Rabbit classI and classII MHC gene haplotypes were described (LeGuern *et al*, 1987; Marche *et al*, 1989). Rabbits (274) belonging to 11 breeds were typed, through RFLP of genomic DNA, for two genes of class II (DQα and DRα). The results are summarised in Table 7.

Table 7. Parameters of genetic variability for MHC haplotypes

Breed	N	A	He	Ho
Argenté de Champagne	27	5	0.725	0.716
Belgian Hare	22	4	0.623	0.500
Chinchilla	22	3.5	0.603	0.532
English	34	3	0.550	0.429
Fauve de Bourgogne	38	4.5	0.544	0.494
French Lop	21	4.5	0.674	0.688
Hungarian Giant	17	4	0.721	0.757
Himalayan	20	3.5	0.589	0.525
Gigante de España	24	3.5	0.540	0.363
Thuringer	26	3	0.243	0.271
Vienna White	23	4.5	0.725	0.479
Mean values		3.9	0.594	0.523

(N, A, He, Ho : see table 3)

A high and significant (SD = 0.035) intra-breed structuring was observed (F_{IS} = 0.13). When the data were considered breed by breed, only the Vienna White breed showed a significant (P≤ 0.001) heterozygosity deficiency. A high and significant (SD = 0.023) inter-breed structuring was also observed (F_{ST} =0.23).

5.2 Antibody Loci (IG loci) (VUB, Belgium)

There is strong evidence that in the rabbit species, allele diversity at the Ig loci is a crucial component of population fitness (van der Loo and Verdoodt, 1992; van der Loo, 1993). Allelic polymorphism was analysed at four loci, each of which contributes to the major fraction of circulating antibodies (IGG) in the rabbit. These loci, and their gene products, are :
- IGKC1 (alias: b locus): Constant region of the predominantly expressed of the IG L chain (Kappa1 subclass).
- IGVH1 (alias: a locus): Predominantly expressed variable region gene of the IG H chain (VH1 gene)
- IGGH (alias: d locus): Hinge region of the H chain constant region of the (sole) rabbit Gamma class.
- IGGCH2 (alias: e locus): CH2 domain of the Gamma H chain.

The IGVH1, IGGH and IGGCH2 loci are closely linked, allowing to distinguish the H chain haplotypes (IGH haplo). Alleles of the L chain locus IGKC1 segregate independently from

those of the H chain. The truly allelic nature of each of these polymorphisms is firmly based upon extensive breeding studies and on genomic analysis. The analysis of variance was carried out by subdividing the rabbits according to breed. Some of them were furthermore subdivided according to geographical origin. Their initials and sample size are shown in Table 8, together with the heterozygosity levels, and the number of alleles at each of the four loci.

Table 8. Parameters of genetic variability for antibody (IG) haplotypes.

Breed	Origin		IGKC1			IGVH1			IGGH			IGGCH2			IGH haplo
		N	He	Ho.	A.	He	Ho.	A.	He	Ho.	A.	He	Ho.	A.	A.
C77	FR	25	0.039	0.040	2	0.039	0.040	2	0	0	1	0.039	0.040	2	3
AC	FR	27	0.172	0.111	3	0.514	0.444	3	0.466	0.370	2	0.036	0.037	2	6
BH	FR	20	0	0	1	0.480	0.600	2	0.095	0.100	2	0	0	1	3
BH	IT	4	0	0	1	0.375	0	2	0.500	0.500	2	0	0	1	3
CH	SW	21	0.210	0.238	2	0.541	0.286	3	0	0	1	0	0	1	3
CH	FR	12	0.469	0.583	2	0.080	0.083	2	0	0	1	0.153	0.167	2	3
EN		38	0.483	0.500	2	0.322	0.290	3	0.422	0.290	2	0	0	1	4
FB	FR	33	0.298	0.303	2	0.213	0.121	2	0.434	0.455	2	0	0	1	3
FB	IT	9	0.000	0.000	1	0	0	1	0.444	0.444	2	0	0	1	2
FG	IT	7	0.337	0.429	2	0.500	0.429	3	0.133	0.143	2	0	0	1	4
FL	HU	11	0	0	1	0.500	0.455	2	0	0	1	0.165	0.182	2	3
FL	FR	16	0	0	1	0.430	0.500	2	0.264	0.313	2	0	0	1	3
HG		17	0.161	0.177	2	0.504	0.706	3	0.457	0.588	2	0	0	1	4
HI	SW	15	0.064	0.067	2	0.464	0.600	2	0.320	0.400	2	0.391	0.400	2	4
HI	FR	12	0.080	0.083	2	0.542	0.333	3	0.153	0.167	2	0.080	0.083	2	5
TH	FR	41	0.156	0.122	2	0.314	0.244	2	0.329	0.220	2	0.329	0.415	2	4
TH	IT	9	0	0	1	0.401	0.556	2	0.401	0.556	2	0.401	0.556	2	3
VW		33	0.088	0.091	3	0.463	0.394	3	0	0	1	0.140	0.152	2	4
Total		350	0.211	0.174	3	0.527	0.331	3	0.344	0.234	2	0.113	0.114	2	7

(N, A, He, Ho : see table 3)

Most, but not all, allotypes known in domestic rabbits and in wild population of the “recent” range (France, Benelux, Great Britain, Australia and Kerguelen) were observed on the whole set of breeds under study. There was a rather dramatic reduction in IG gene diversity in the present sample when compared to wild populations (van der Loo, 1993). One allele was completely missing (IGKC1-b9 allotype) and two were very rare (IGVH-a2 and IGGC2-e14). In contrast, one allele at IGKC1 locus (b6) was found more frequently than in most published studies on wild populations. Some “common” H chain haplotype combinations were absent or rare, although one haplotype was observed that so far has not been reported in the laboratory rabbits (the IG[VH1-GCH2] combination a3-e14).

The analysis of variance (Table 9) indicates that, for the IGVH1 and IGGH loci, a large part of the diversity in the total sample was due to differences among breeds (i.e. F_{ST} values are large) and inbreeding is significant (F_{IS} positive). In contrast, these population parameters were lower or even negative at IGKC1 and IGGCH2 loci. There are indications that these differences have a biological meaning. Indeed, previous studies have revealed significant linkage disequilibria between these two independently segregating loci (van der Loo *et al*, 1996). This highly unusual situation is most likely due to the fact that both loci (which control the H-chain and L-chain constant region) are subjected to (compensatory) overdominance

selection (van der Loo *et al,* 1996) i.e. heterozygosity is favoured at IGKC1 or at IGGCH2. Available data on more than 8000 wild rabbits from the recent species range (France, Benelux; Great Britain, Australia and Kerguelen) have shown that F_{IS} values at these interacting loci are consistently lower when compared to those at other loci. Unlike in this study, differences in F_{IS} values among loci were highly significant in the studies on wild populations (van der Loo 1993, van der Loo *et al* 1996). They suggest selection forces of the order of 20%, which is indicative of foetal-maternal interaction (Otha, 1998). The conditional probability that the lower than average F_{IS} values at these loci are due to chance is therefore low (Table 9).

Table 9. F-statistics for the antibody (IG) loci

locus	F_{IS}	F_{ST}
IGKC1	0.011	0.173
IGVH1	0.114	0.306
IGGH	0.089	0.266
IGGCH2	-0.146	-0.125
IGHaplo	0.086	0.269

In this context, it is important to note that the reduction in gene diversity in the breeds under study is pronounced at precisely the IGKCI and at the IGGCH2 loci (Table 10), with a number of breeds being fixed for one of their alleles (BH is fixed for both).

Table 10. Total heterozygosity (*HT*) in RESGEN and in wild populations

HT	*IGKC1*	*IGVH1*	*IGGH*	*IGGCH2*
RESGEN breeds	0.211	0.527	0.344	0.113
Wild Europe	0.536	0.628	0.350	0.341
Wild G-Britain	0.535	0.586	0.402	0.327
Wild Australia	0.421	0.577	0.387	0.320
Kerguelen	0.001	0.474	0.192	0.404

In summary, the present analysis showed that, except for one IGKC1 allele, all allotypes, known to occur in domestic rabbits were present in this sample. However, in most breeds some of these alleles were missing, and the average heterozygosity level was significantly lower than in wild rabbits. In agreement with previous population studies, the F_{IS} values tended to be lower at the IGKC1 and IGGCH2 loci, when compared to F_{IS} values at other IG loci and were also lower than those at most other loci studied in this project. The data obtained should contribute to the preservation of IG polymorphisms in the selected breeds, which, in view of evidence of the adaptive importance of population diversity at the rabbit IG loci, may be of great importance.
Since there is evidence of the adaptive value of polymorphism at some IG loci, and since in many of the individual breeds these polymorphisms are endangered or lost, the preservation of rare IG alleles should be emphasised in any rabbit conservation program.

EVALUATION OF BREED VARIABILITY FOR ZOOTECHNICAL TRAITS

Very few data exist on zootechnical performances of original breeds or local populations in Europe. Most previous work has been devoted to selected strains or crossbreeding (see

Rochambeau, 1988, for a review), and comparative studies of native breeds are lacking. Only some zootechnical evaluations so far have concerned a few breeds treated separately (Rouvier, 1970; Ouhayoun, 1983; Pilandon *et al*, 1986; Chevalier *et al*, 1986; Grandi and Stefanetti, 1987; Lopez *et al*, 1992; Pagano Toscano *et al*, 1992; Koehl and Van Der Horst, 1998), and some crossbred products of these breeds have been compared (Ouhayoun and Poujardieu, 1986). A research network on rabbit production in the Mediterranean area is currently working on the identification and characterisation of local populations and breeds. Southern Mediterranean populations have been considered (Rouvier, 1994; Khalil, 1998).
Within this program, the zootechnical performances were evaluated :
- in three experimental farms, in comparison with the control strain (France : CEZ at Rambouillet and INRA at Auzeville, Italy : ISZ at Torino). Each breed was evaluated in two different farms,
- at UNIZAR (Spain) for the AC and FB breeds, without control strain,
- at KATKI (Hungary) for the Hungarian Giant breed (not presented here).
The experimental procedures have been described by Bolet *et al* (1999). The evaluated traits were :
- reproductive traits : Reproductive performances of founder females (G0) and those of their daughters (G1) were recorded. G1 females were compared with contemporaneous C77 does. The traits analysed appear in Table 11.
- growth traits : After weaning, growth and food consumption (not presented here) of G1 does progeny were recorded until the progeny were 11 or 12 weeks old.
- carcass traits : Rabbits were slaughtered at 11 or 12 weeks and carcass was submitted to a standardised dissection (Blasco *et al*, 1993). Muscle samples were taken to characterise the fibre types and meat composition.
Here, we only present a preliminary part of the results.

1. Reproductive traits

1.1 Material and methods

This evaluation concerned 8 breeds out of the 10 to be evaluated (the giant breeds, FG and FL, have not yet been evaluated) on 2 experimental farms at INRA (Auzeville) and CEZ (Rambouillet). It covered a 30 month period, between July 1997 and December 1999. There were 2 different evaluation batches in Auzeville. The first one included the AC, BH, EN, FB and VW breeds; the second one included the AC, CH, EN, HI, TH and VW breeds. The single evaluation batch in Rambouillet concerned the CH and HI breeds. The animals were kept in so-called 'rational' buildings equipped with wire cages. They were fed commercial pelleted diets *ad libitum*. Reproduction started at the age of 18 weeks. Artificial insemination was the predominant reproduction method in Auzeville while natural mating was used in Rambouillet. Does were mated or inseminated 12 days after kidding to achieve a semi-intensive reproduction system. Weaning occurred about 35 days after birth.
The traits analysed concerned doe fecundity and litter traits (Table 11). The statistical model of analysis included the fixed effects of breed, group (a combination of experimental station and of batch), age of the does (4 classes) and season (4 levels).

1.2 Results

Fertility rates were highly variable, the overall mean being low. TH, VW, AC and BH had the lowest fertility rates, ranging from 25.6% to 42.9%. HI, EN and CH which are small body

sized breeds, confirmed the reputation of such types to be fertile, but had lower fertility rates than the control strain.
On the whole, the breeds studied had smaller litter sizes than those of the control strain, either at birth or at weaning. The difference between the average of the breeds studied and the control was about 2 rabbits. To simplify, we can define two groups of breeds based on the average litter size at birth : the first one includes the BH, FB, VW and CH breeds, the second one includes the TH, EN, HI and AC breeds, whose litter size was still lower.

Table 11. Reproductive performances of 8 breeds compared to the C77 control strain (preliminary results from 8 breeds)

	C77	AC	BH	CH	EN	FB	HI	TH	VW
					Fecundity of does				
Does weight at mating (g)	4065^{c}	4668^{a}	3563^{d}	3454^{e}	2972^{f}	4100^{bc}	2689^{g}	4189^{b}	4140^{b}
Fertility (%)	68.9^{a}	37.5^{de}	42.9^{cd}	61.2^{b}	60.7^{b}	50.7^{c}	70.6^{a}	25.6^{e}	32.5^{e}
litters with 0 born alive (%)	9.0^{a}	22.9^{b}	12.1^{ab}	8.4^{a}	19.9^{b}	20.0^{b}	19.6^{b}	16.6^{ab}	16.6^{ab}
Total born	7.24^{a}	4.77^{cd}	6.13^{b}	5.22^{cd}	4.93^{cd}	5.54^{bc}	4.78^{d}	4.91^{bcd}	5.50^{bc}
Born alive	6.26^{a}	3.45^{c}	4.21^{bc}	4.53^{b}	3.62^{c}	4.23^{bc}	3.59^{c}	3.8^{bc}	4.38^{bc}
Stillborn	0.98	1.33	1.92	0.70	1.31	1.32	1.20	1.05	1.13
					Litter traits				
Litter weight at birth (g)	517^{a}	287^{cd}	327^{bc}	365^{b}	204^{e}	267^{d}	289^{cd}	278^{bcde}	271^{d}
IWB[1] (g)	79^{a}	73^{bc}	74^{bc}	78^{ab}	58^{d}	61^{d}	71^{c}	71^{bc}	62^{d}
Litter size at weaning	6.23^{a}	3.96^{bcd}	3.03^{d}	4.45^{b}	3.81^{cd}	4.69^{b}	4.36^{bc}	3.79^{bcd}	4.16^{bc}
Dead kids B-W[1]	0.6	0.8	2.5	0.7	1.1	1.2	0.3	1.2	1.9
Litter weight at weaning	5304^{a}	4005^{b}	2798^{d}	3245^{cd}	2491^{d}	3532^{bc}	2863^{d}	4023^{b}	3120^{cd}
IWW[1] (g)	900^{b}	1090^{a}	957^{b}	763^{d}	734de	838^{e}	701^{c}	966^{b}	800^{cd}
ADG B-W[1] (g/d)	24.4^{b}	28.7^{a}	24.4^{ab}	19.6^{cd}	18.5^{d}	22.2^{bc}	18.2^{d}	24.5^{abc}	21.0^{bcd}
Dead litters B-W[1] (%)	17.6^{a}	38.5^{bcd}	44.7^{cd}	27.4^{b}	46.8^{d}	35.5^{bc}	35.9^{bc}	31.2^{abcd}	51.7^{d}

Within lines means with different letters are significantly different ($P<0.05$).
[1] IWB=average individual weight at birth; ; bB&W = between birth and weaning; IWW=average individual weight at weaning; ADG=average daily gain.

Total mortality at birth includes two phenomena : mortality of the whole litter and mortality "by unit" of only some kids in the litter. The total number of stillborn rabbits, including both types of mortality, was 1.1 on average for all breeds. The highest values were 1.9 for the BH breed, 1.3 for the AC, EN and FB breeds and 1.2 for the HI breed. Except for the BH breed, these high mortalities were due to the mortality of entire litters, at a high rate of about 20%. In breed BH, "by unit" mortality was predominant. The CH breed displayed the lowest mortality at birth (0.7 stillborn). The rate of mortality of entire litters was 14% on average. This rate was the lowest in the control strain and CH (about 9%). It reached about 20% in the AC, EN, FB and HI breeds.
At weaning, the average litter size for all the breeds studied was about 4. On the whole, the ranking of breeds according to litter size at weaning was more or less the same as at birth. Three breeds underwent a dramatically high mortality rate of entire litters between birth and

weaning : VW (51.7%), EN (46.8%) and BH (44.7%). In two of them, an additional "by unit" mortality was recorded, 2.5 and 1.9 rabbits in the BH and VW breeds respectively. The CH and TH breeds exhibited the lowest rate of litter mortality (27.4% and 31.2% respectively), associated with a low by unit mortality in the CH breed (0.7 rabbits dead). The HI breed showed the lowest 'by unit' mortality.

2. Growth rate after weaning

Table 12. Analysis of the growth curves on the 4 experimental farms : coefficients of regression of live weight and relative growth rate against age

Trait			Live weight		Relative growth rate			
			linear coefficient		linear coefficient		quadratic coef.	
Auzeville								
R2 model			0.829		0.442			
Breeds	C77	72	36.04		-0.2120		0.001149	
	AC	44	36.84	+	-0.1825	+	0.000762	-
	BH	30	32.96	-	-0.2175	=	0.001191	=
	CH	47	29.77	-	-0.2225	=	0.001295	=
	EN	113	26.19	-	-0.2150	=	0.001193	=
	FB	123	33.54	-	-0.1975	+	0.000979	-
	HI	75	26.26	-	-0.2423	-	0.001561	+
	VW	43	31.69	-	-0.1965	+	0.000978	=
Rambouillet								
R2 model			0.851		0.427			
Breeds	C77	68	34.24		-0.2315		0.001343	
	CH	194	29.29	-	-0.2210	=	0.001242	=
	HI	173	22.78	-	-0.2682	-	0.001847	+
Torino								
R2 model			0.872		0.495			
Breeds	C77	21	34.75		-0.1936		0.001156	
	C77X	29	34.50	=	-0.1739	+	0.000863	-
	BHX	51	36.21	+	-0.1744	+	0.000901	-
	FBX	48	37.65	+	-0.1956	=	0.001141	=
	FG	23	40.79	+	-0.1539	+	0.000631	-
	FGX	35	39.07	+	-0.1676	+	0.000806	-
	THX	51	36.18	+	-0.1792	+	0.000973	-
Zaragoza								
R2 model			0.806		0.467			
Breeds	AC	31	37.62	a	-0.2762	a	0.001343	a
	FB	13	33.49	b	-0.3090	b	0.001242	b

Within farms, '=': not different from the C77; '+'or '-': greater or lower than the C77; a>b ($P<0.05$)

A total of 1284 young rabbits, raised in single or collective cages and fed *ad libitum*, were weighted weekly from weaning until the age of 11 or 12 weeks on the experimental farms of Auzeville (France), Rambouillet (France), Torino (Italy) and Zaragoza (Spain). They belonged to 14 genetic types : 8 original breeds plus the control (C77, AC, BH, CH, EN, FB, FG, HI, VW) and 5 crossbred types, specific to Torino (C77X, BHX, FBX, FGX, THX). The latter were produced by crossing sires of the studied breeds with a New-Zealand White dam strain from Torino.

On the one hand, individual weights (W, g) were regressed against the corresponding age (t, days) to obtain average daily gain (dW/dt) per breed and farm. On the other hand, the relative growth rate (dW/W, expressed as percent of weight gain per day calculated per period) was fitted against the age by quadratic regression; the linear coefficient represents the rate of decrease of the relative growth rate; the quadratic coefficient represents the rate of deceleration of the relative growth rate, thus an higher positive value means delayed decline in relative growth rate and *vice-versa* a lower positive value.
Growth curves showed interesting features which can be discussed according to the live weight wide range (Table 12). In Torino, either purebred or crossbred Flemish Giant (adult weight of standard females >6 kg) grew faster than control rabbits (C77) showing a slower decline of the relative growth rate. Among the medium sized breeds, only the Argenté de Champagne and the crossbred rabbits from Torino grew faster than the control strain. The Belgian Hare and Vienna White breeds exhibited the slowest growth rate. Fauve de Bourgogne, Argenté de Champagne and Vienna White breeds gave equivalent figures in decreasing their relative weight gain at a delayed age, because the coefficients are similar. The small sized breeds (Chinchilla, Himalayan and English) had a 25-35% slower growth rate than the control. However, the parameters of their relative growth rates were identical to the control, except for the Himalayan, which showed a faster decreasing relative growth rate.

3. Carcass traits

Table 13. Partial results of carcass traits

	80 d live weight (g)	Hot dressing %	Liver (%)[1]	Perirenal Fat (%)[1]	Scapular Fat (%)[1]	Head (%)[1]	Hind leg meat/bone ratio	pH Ld[2]	pH Bf[2]
					Auzeville				
C77	2755 ^{a}	61.6 ^{c}	5.75 ^{a}	3.21 ^{a}	0.99 ^{a}	7.6 ^{b}	5.46	6.01 ^{abc}	6.15 ^{b}
AC	2865 ^{a}	64.1 ^{ab}	5.30 ^{b}	2.16 ^{c}	0.64 ^{b}	7.2 ^{c}	5.18	6.10 ^{ab}	6.22 ^{ab}
BH	2454 ^{c}	64.3 ^{a}	4.51 ^{c}	1.31 ^{d}	0.33 ^{d}	8.0 ^{a}	4.49	6.13 ^{a}	6.30 ^{a}
CH	2275 ^{d}	61.6 ^{c}	4.08 ^{c}	0.98 ^{d}	0.39 ^{cd}	8.0 ^{a}	5.26	5.97 ^{abc}	6.23 ^{ab}
EN	1912 ^{e}	63.2 ^{b}	5.18 ^{b}	2.43 ^{bc}	0.62 ^{b}	8.0 ^{a}	5.72	5.98 ^{c}	6.18 ^{ab}
FB	2622 ^{b}	63.2 ^{ab}	5.52 ^{ab}	2.58 ^{b}	0.48 ^{c}	7.4 ^{bc}	5.19	5.99 ^{abc}	6.12 ^{b}
VW	2422 ^{c}	61.6 ^{c}	5.51 ^{ab}	2.53 ^{bc}	0.72 ^{b}	8.1 ^{a}	5.51	5.91 ^{c}	6.10 ^{b}
					Torino				
C77	2352 ^{cd}	58.3 ^{d}	5.84 ^{a}	1.28 ^{bc}	0.46 ^{b}	9.6 ^{a}	6.12 ^{ab}	5.77 ^{cd}	5.89 ^{b}
C77X	2306 ^{d}	60.3 ^{c}	5.99 ^{a}	1.40 ^{ab}	0.56 ^{a}	9.2 ^{ab}	6.49 ^{a}	5.81 ^{bc}	5.92 ^{ab}
BHX	2465 ^{c}	60.8 ^{c}	5.88 ^{a}	1.21 ^{bc}	0.50 ^{ab}	8.9 ^{bc}	6.30 ^{ab}	5.79 ^{cd}	5.89 ^{b}
FBX	2456 ^{c}	62.0 ^{ab}	4.91 ^{b}	1.13 ^{c}	0.35 ^{c}	9.4 ^{a}	6.52 ^{a}	5.75 ^{d}	5.82 ^{c}
FG	3126 ^{a}	61.2 ^{bc}	5.55 ^{b}	1.22 ^{bc}	0.46 ^{ab}	8.6 ^{c}	5.88 ^{b}	6.04 ^{a}	6.07 ^{a}
FGX	2861 ^{b}	62.2 ^{ab}	4.71 ^{b}	1.30 ^{bc}	0.46 ^{b}	8.8 ^{c}	6.14 ^{ab}	5.85 ^{b}	5.95 ^{a}
THX	2456 ^{c}	62.3 ^{a}	5.72 ^{a}	1.57 ^{a}	0.53 ^{a}	8.7 ^{c}	6.50 ^{a}	5.78 ^{cd}	5.88 ^{b}

[1] In percent of commercial carcass weight (Blasco *et al*, 1993)
[2] Ld = Longissimus dorsi, Bf= Biceps femoris; By farms, a>b>c>d ($P<0.05$);

A sample of 290 rabbits of 6 breeds plus the control from Auzeville and 248 from Torino, representing 7 genetic types were analysed. The rabbits were slaughtered at two fixed ages, 77 and 84 d, after electric stunning. Carcasses were refrigerated for 24h at 4°C. The carcass composition was studied according to classical criteria defined and harmonised by Blasco *et al* (1993). Table 13 indicates partial results obtained in Auzeville and Torino respectively.

All traits studied except the muscle/bone ratio of the hind leg in Auzeville, exhibited significant variation between breeds or types. From the Auzeville results, dressing percentage was maximum in BH and AC and minimum in the CH, VW breeds and the control. Surprisingly, variations in liver and fat proportions appeared to be correlated: C77 displayed the highest values for both traits, breeds CH and BH the lowest values. With regards to muscle proportion in the carcasses, estimated by the ratio muscle/bone in the hind leg, the maximum occurred in the EN breed, followed by VW and C77. As expected from its slender body shape, the BH breed had a low ratio.
The results from Torino concerning the dressing percentage showed that TH and FB crosses performed better than BH crossbreds. The proportion of liver, a valuable cut, was high in the crossbreds from C77, BH and TH. The FG pure-breds and crossbreds and the TH crosses differed by a low proportion of their heads in the carcasses. The FG pure-breds were characterized by a lower meat contents in the hind leg and their meat appeared less acidified as shown by a high ultimate pH. According to the fat deposits, FBX appeared as a very lean breed, leaner than BHX which was not the case for the corresponding pure-breds in Auzeville.

4. Meat quality traits

4.1 Muscular fibres analysis

This study concerned the percent of type I (slow twitch) and type II (fast twitch) fibres in *Biceps femoris* (Bf) and *Longissimus dorsi* (Ld) from rabbits slaughtered at 11 or 12 weeks of age. A total of 113 rabbits from six breeds (C77, AC, BH, EN, FB and VW), i.e. a small part of the total sample under study in this program, were studied in Zaragoza. An immunocytochemical technique using monoclonal antibodies against fast myosin was used; it allowed to distinguish and to count type I fibres, (which do not become coloured) from type II ones (which become coloured). In the following text, only the percent of type I fibres will be presented, the type II percent being the complement to 1. Percents were analysed using an analysis of variance with the fixed effects of breed, age (11 or 12 weeks), sex and all possible interactions between the latter effects.

Table 14. Percentage of type I fibres in *Longissimus dorsi* according to breed and age (standard deviation in brackets)

Age (weeks)		C77	AC	BH	EN	FB	VW
11	Mean	4.96	8.32	5.54	4.93	4.81	5.52
	(s.d.)	(1.88)	(3.23)	(0.95)	(1.32)	(1.40)	(1.52)
12	Mean	4.76	4.30	5.67	4.12	5.34	2.70
	(s.d.)	(1.59)	(0.56)	(2.32)	(0.85)	(1.48)	(0.42)

The percentage of type I fibres were very similar in both muscles : 4.88% (± 1.98) in Bf and 5.11% (±1.77) in Ld. These values were similar to those observed by other authors for the same muscles (Lambertini *et al*, 1996; Lobley *et al*, 1977; Vigneron *et al*, 1976). However, the pattern of variation resulting from the analysis of variance appeared quite different for the 2 muscles : In Bf, none of the factors in the variance analysis influenced the fibre type composition. In Ld, age and breed by age interaction (Table 14) appeared statistically significant ($P<0.01$).
The percentage of type I fibres decreased between the age of 11 and 12 weeks in all breeds except FB and BH. The decrease was more marked in the AC and VW breeds. Although Lambertini *et al* (1996) did not find any influence of age on the percentage of fibre types,

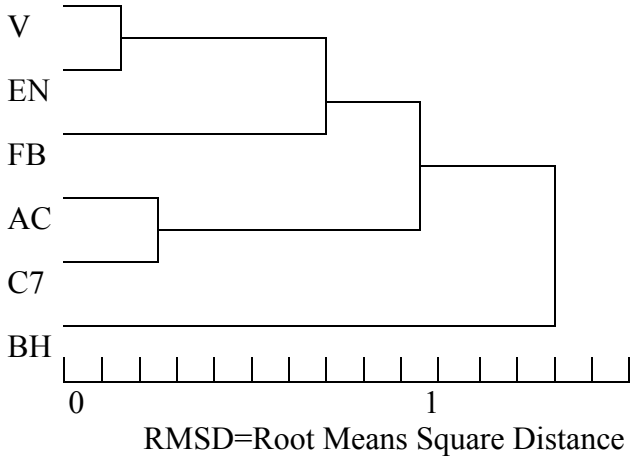
other authors like Lobley *et al* (1977) observed a decrease with age in type I homologous fibres in muscles having a similar fibre composition as Ld. This age related increase in type II fibres proportion has been shown to be related to a decrease in muscular pH (Gondret and Bonneau, 1998) and a change in correlated traits (longer conservation lag, clearer meat, less water retention,…). However, the change in fibre type proportions in our results was so slight that no correlated effect was expected; moreover, these results concerned a low number of samples, mainly for AC and VW breeds, so they must be considered with caution.

4.2 NIRS (Near Infrared Reflectance Spectroscopy)

Table 15. Characteristics of the 150 animals under NIRS examination

Breeds	C77	AC	BH	EN	FB	VW	R^2	Prob.
N	32	11	16	28	48	15		
Age, d	82±4	81±4	81±4	81±4	81±4	82±4	0.01	0.86
Live weight, g	2716	2843	2418	1802	2588	2467	0.60	0.001
	±344	±368	±221	±233	±253	±264		
Estimated LD lipids	4.9	3.6	1.9	3.1	4.0	2.8	0.35	0.001
	±1.6	±1.1	±0.7	±0.8	±1.3	±1.2		
Estimated HL lipids	11.1	10.7	10.2	11.3	11.9	11.5	0.02	0.63
	±4.0	±4.4	±3.1	±3.6	±3.3	±2.6		
Estimated OA lipids	26.4	24	11.9	17.6	29.2	24.1	0.33	0.001
	±6.8	±9.80	±6.2	±7.3	±9.3	±10		

Figure 1. Dendrogram of the breeds according to NIRS matrices of distances.



4.3 Other meat quality traits

A sample of *Longissimus Dorsi* collected on the cold carcass, stored at -20°C was controlled in Monterotondo (Rome, Italy) for some other meat quality traits: After thawing, raw shear force (RWB) and color (L, a, b) both on raw and cooked meat were recorded (Bergoglio *et al*, 1997). The first results are given in Table 16. Favourable trends for raw meat tenderness were evidenced for Argenté de Champagne, English and Vienna White, but not for cooked meat. Differences in colour were enhanced in frozen then thawed meat, before cooking: a reduced lightness (L*) was ascertained in the Belgian Hare and in the English. The latter also showed a reduced redness value (a*) of raw meat.

Table 16. Preliminary results of meat quality traits (Auzeville's first 6 breeds; N=80)

	C77	AC	BH	EN	FB	VW
N	14	3	7	17	30	9
Thaw loss (%)	8.70	7.68	8.36	7.98	7.95	8.17
Cooking loss (%)	23.78[a]	22.78[b]	23.39[a]	23.09[b]	22.90[a]	24.10[b]
RWB raw meat	15.88[b]	12.53[b]	16.51[b]	13.66[a]	16.07[b]	13.54[ab]
RWB cooked meat	25.45[ab]	21.36[a]	25.06[b]	33.26[b]	26.33[ab]	28.61[a]
L_raw	59.39[ab]	60.90[a]	58.05[ab]	58.37[b]	59.68[a]	60.13[ab]
a_raw	4.93	6.07	5.37	4.45	5.74	5.04
b_raw	14.44	15.02	14.10	14.63	15.07	14.67
L_cooked	77.34	79.17	76.81	77.53	77.66	78.34
a_cooked	1.40[ab]	0.95[b]	1.49[a]	1.10[b]	1.23[a]	1.25[a]
b_cooked	15.23[a]	14.24[c]	15.67[d]	15.78[c]	14.93[b]	15.61[c]

a>b>c>d RWB : Raw Shear Force

CRYOCONSERVATION OF SEMEN AND EMBRYOS

A European concerted action ("germplasm banking"), in which the rabbit was used as a model animal, allowed the definition of the technical basis for cryoconservation of rabbit embryos. Embryos from some commercial crossbred strains and from 3 small size endangered strains were collected and frozen (Joly *et al*, 1994, 1996). Except this case, however, there is no systematic collection of rabbit genetic resources, as exists in most other domestic animal species Their preservation is currently performed *in situ* by either professional or fancy breeders, but without any consistent program such as that of the "Conservatoire National des Animaux de Basse-Cour" which existed in France from 1978 to 1985 (Arnold and Rochambeau, 1983) and now no longer exists. The setting up of *in situ* conservation will have to be discussed in view of the definite results of this program. But a first step in conservation consists in freezing semen and embryos from the animals used in this program, which are now well characterised from the genetic and zootechnical point of view. To date, cryopreservation of gametes and embryos consolidates the application of animal breeding in a wide range including genome cryobanking (Joly *et al*, 1996) to an unlimited date. Methods for freezing semen and embryos in rabbit species have now reached a sufficient efficiency so that they can be applied in cryobanking programs.

1. Material and Methods

Female and male rabbits of 8 breeds (see above) were used for either embryo production or semen collection. They were either founder animals (G0) or progeny (G1). The collected embryos and semen were cryopreserved and stored for cryobanking. Semen cryopreservation was performed in a sucrose-DMSO extender analogous to the technique described by Viudes de Castro and Vicente (1996). Prior to embryo collection, donor animals were either treated with FSH, or PMSG, to induce superovulation, or they did not receive any superovulation treatment. Ovulation was induced by either GnRH or hCG and donors were simultaneously inseminated. Morulae and blastocysts were either flushed from oviducts and uterine horns of slaughtered animals, or collected by endoscopy (Besenfelder *et al*, 1998). Cryopreservation was performed using a slow freezing technique (Joly, 1997). Embryos were plunged during 5 minutes each time in 3 successive baths of PBS containing respectively 0.5 M, 1.0 M, and 1.5

M DMSO. Embryos were seeded at –7 °C followed by freezing to – 30 °C (0.5 °C/min) and direct plunging into liquid nitrogen.

2. Results

2.1 Semen preservation

Twenty-nine animals from 7 different breeds delivered enough semen for cryopreservation in 361 straws. Sperm motility, acrosomal integrity, and abnormal spermatozoa varied between 50 – 90 %, 47 – 100 %, and 4 – 47 %, respectively. The variations of measured traits were observed for individual animals rather than for the different breeds (Table 17).

Table 17. Number of semen cryoconserved straws(preliminary results with 5 breeds)

Breed	Males	Number of straws	Min-max per buck	Sperm motility
Argenté de Champagne	2	28	9-19	
Chinchilla	5	51	4-17	50 – 80 %
English	5	73	5-37	70 – 90 %
Fauve de Bourgogne	2	11	5-6	
Himalayan	4	62	6-23	60 – 90 %
Thuringer	7	71	4-25	60 – 90 %
Vienna White	4	65	9-33	60 – 90 %
Total	29	361		50 – 90 %

2.2 Embryo cryopreservation

A total of 132 animals were used for single or repeated embryo recovery. These donors belonging to 8 breeds delivered 1471 embryos which were considered as suitable for cryopreservation. With exception of English, Himalayan, Vienna White and Chinchilla rabbits, superovulation treatment did not result in an extra number of embryos (Table 18).

Table 18. Number of females and cryoconserved embryos (preliminary results with 8 breeds)

Breeds	Donor females	Stored embryos
Argenté de Champagne	4	52
Belgian hare	10	127
Chinchilla	27	260
English	21	286
Fauve de Bourgogne	4	28
Himalayan	32	327
Thuringer	9	59
Vienna White	25	332
Total (8 breeds)	132	1471

3. Discussion

The aim of the present project was to collect and cryopreserve semen and embryos of different breeds which had been housed and bred before in order to perform zootechnical

evaluation and genetic characterisation. It was shown that semen and embryo collection including cryopreservation of different breeds can be performed successfully. Although breed-specific limitations were noted, initial work in this field resulted in almost 1500 frozen embryos and some amounts of frozen semen.

FIRST INFERENCES

In the previous chapters, we presented the first results of an ambitious program of inventory of European rabbit breeds on the one hand, evaluation and conservation of some of them on the other hand. This program is still in progress and all the analyses have not been completed. So these are preliminary results and a complete and global analysis remains to be done. We can, however, already draw some inferences from these results, concerning the specific problems faced at each step and the implications for new developments.

1. Specific questions

1.1 Inventory of European breeds

The motivation of breeder national associations was the limit to the efficiency of the constitution of the data bank. In spite of the efforts of the French federation and the European association of rabbit breeders, the number of countries which filled in the questionnaire was rather low. The efforts of the French FAO focal point did not increase the information feedback very much. However, we got around 150 answers, which allowed to initiate the computerised data bank. It is now a living instrument which has to be continuously fed and updated. Its introduction into international FAO and EAAP data banks will be an important step for its diffusion.

1.2 Choice of breeds and sampling of animals

To choose the breeds to be evaluated, we did not take into account the risk of extinction of the breeds, but their historical status and their potential zootechnical interest. Under these conditions, the choice was rather easy, but the main problem was the sampling of the animals. We had to restrict the purchasing of animals to some of the countries interested in the program, namely France, Italy, Hungary and Switzerland. However, in spite of these restrictions, and given the importance of pure breeding in these countries, we consider that the sampling was rather satisfactory, as far as a global description of each breed is concerned. We tried to buy animals that were as unrelated as possible, but Table 1 shows that this objective was only partly reached. The number of sires per breed was mostly around 15-20, which is slightly inferior to classical recommendations.

1.3 Evaluation of genetic diversity

FAO published recommendations about the choice of markers for the evaluation of genetic diversity of breeds (Barker *et al*, 1998). It was not possible to apply them, because we know very little about molecular genetics of the rabbit (Van Haeringen *et al*, 1997). But this was compensated by the great diversity of the markers used. There was generally a good agreement between the results from the different markers. Rather high values were obtained for F_{ST}, which indicates a strong differentiation between breeds. However, the rather high values obtained for F_{IS} could be relevant of strong relationship between animals, due to the

sampling and/or genetic structuring within a breed. This point has to be examined more in detail; however, a preliminary analysis showed that limiting the sample to not closely related animals did not change the F_{ST} and F_{IS} values. This result favours an origin of high F_{IS} from the way breeds are organised. Indeed some genetic structuring might exist within breeds due to low exchanges of animals between breeders. The complete analysis of all the markers together and the calculation of genetic distances are necessary before concluding.
Any time comparisons with wild populations could have been done, the level of breed diversity was found far lower than that of wild populations from the original range of the species (Iberian Peninsula) but almost the same as in more recent populations (see Callou, 1995 for further details on rabbit history).

1.4 Evaluation of zootechnical performances

It was quite impossible to collect valuable zootechnical data under farming conditions. So the only source of information is the comparison of these breeds on different experimental farms with a common control strain. The results given here are only partial, but they indicate very poor reproductive performances on all experimental farms (see Table 11). We can imagine that the choice of the rearing system was not well adapted to these breeds; besides, transportation of rabbits after weaning from different breeders to one place may induce hygienic problems and breeding difficulties. So, the results concerning reproductive performances have to be considered with caution. Data obtained concerning growth, carcass and meat quality, seem to be more reliable and interesting, because of the great variability of some traits and the specificity of some breeds, such as the Belgian Hare, for example. There is no doubt about the potential interest of some of these breeds, and the necessity of further research in this respect.

1.5 Conservation

The constitution of a cryobank of embryos and semen is only one aspect of a conservation program. In the case of the breeds involved, we can consider that they are not in danger of extinction; this cryobank is rather a bank of well characterized animals, for most of them, from a genetic and zootechnical point of view. They may represent a source of embryos if some of the alleles and haplotypes are proven later to be of interest or rare ; it also constitutes a good image of the state of these breeds nowadays, which could allow comparisons later.
In this program, we did not get into *in situ* conservation, i.e. genetic management of breeds, because an analysis of the genetic situation is necessary, which will be made possible partly by the results. This point is discussed below.

2. Implications and perspectives

2.1 Continuation of the evaluation and other traits to be evaluated

The zootechnical information has to be completed and improved. The first step is to obtain an experimental sample of animals that reflect the diversity of origins but avoid the problems we have met up to now. Since the freezing of embryos and semen has proven to be a valuable technique, we propose to constitute this sample by embryo transfer after having completed the embryo bank. We observed, however, important variations between animals and breeds concerning ovarian response to the superovulation treatment, number and quality of collected embryos, sensitivity to seasonal effects,... In consequence, adaptation of classical treatments to these breeds has to be investigated (Joly *et al*, 1998).

This further evaluation should apply to traits which seem to be of interest in the scope of a zootechnical use of these breeds. We think that, whatever the conditions, reproduction will never be an interesting trait of these breeds. On the contrary, our first results show their great diversity and, in some cases, originality, for carcass and meat traits. This suggests that some of these breeds could be used as sire breeds in crossbreeding. Thus, this evaluation should be carried out both in pure breeding and in crossbreeding, as it has begun on the Torino farm.
Besides, original traits have to be searched. For example, it would be of great interest to evaluate the variability for genetic resistance to diseases.
The genetic evaluation also has to be continued in connection with new knowledge of molecular genetics of the rabbit. These breeds can be an original tool for the research of markers, mainly QTL, for zootechnical traits.

2.2 Combination of genetic and zootechnical information on diversity

One of the main conclusions of this program is that there is both an important between breed genetic diversity and zootechnical, i.e. phenotypic, variability. In the programs of conservation of genetic resources, characterisation of neutral diversity and calculation of genetic distances based on this diversity have become a habitual tool. We think that two points have to be investigated :
- Both genetic polymorphism and phenotypic variability allow to build distances dendograms. It would be of great interest to combine these two kinds of information to better characterise the diversity of breeds. Some statistical methods, such as factorial analysis, should allow to do this and will be used to complete this analysis.
- However, no evidence exists for the relation between the neutral diversity and phenotypic variability. Very few authors have discussed this point (Kremer, 1994; Burstin and Charcosset, 1997) and no strong evidence of this relation exists. Ruane (1999) considered that the relative value of genetic distances for breed conservation is limited. According to Edding and Laval (1999), two populations which are genetically distant need not be phenotypically different. Our data should allow to further investigate this point.

2.3 Conservation

The situation of European rabbit breeds among domestic mammals is unique : there is still a great diversity of breeds, but these breeds are mainly owned by breeders who have more interest in their phenotype for exhibitions than their zootechnical and economical value, so that the genetic management of these breeds is adapted to this objective. The high values obtained for F_{IS} could reflect this uniqueness. The question is whether this management allows to maintain and reinforce the zootechnical originality and potential interest of these breeds. Our opinion is that there is some risk of disappearance of the original zootechnical characteristics of some of these breeds, due to their small effective size (inbreeding, genetic drift). Faced with this risk, we suggest that the breeder organisations take it into account in the genetic management of the breeds and that the sampling of animals and breeds in the cryobank be enlarged.

3. Conclusion

This European program was the first opportunity to approach the evaluation and conservation of rabbit genetic resources in such an extensive way. It is, however, the first step of a process which has to be continued and enlarged. The main features of this process are :

- the recognition of the rabbit as a species of economical interest on the European level and its introduction into data banks on animal genetic resources,
- the synergy between breeder organisations and complementary fields of research, i.e. animal breeding, zootechnics, molecular genetics and population genetics.

The interest for in rabbit breeds by scientists who have been up to now mainly involved in specialised strains or wild populations, is an asset for these breeds which are the reserve of genetic variability of domestic rabbit.

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