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中国茸鹿品种(品系)的随机扩增多态 DNA(RAPD)研究*

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摘 要 采用随机扩增多态 DNA 技术研究了 5 个茸鹿品种(品系)75 个个体的遗传变异关系.在所使用的 41 个引物中,有 40 个引物扩增出多态谱带,共检测到 395 条扩增片段,其中 259 条(65.6%)出现变异.用 Shannon 指数计算了 5 个品种(品系)的遗传变异及其遗传变异在群体内和群体间的分布.利用 Nei 氏片段共享度计算了 75 个个体间的遗传距离,用 UPGMA 聚类法构建了 75 个个体的系统发育树状图,反映 5 个茸鹿品种(品系)的遗传关系.结果表明,遗传变异主要存在于群体内(53.97%),群体间的遗传变异居次要地位(46.05%);双阳品种群体内的遗传变异最低;5 个品种(品系)的遗传多样性指数的大小依次为:西丰(1.124 9)、长白山(1.051 7)、乌兰坝(0.990 8)、清原(0.974 8)、双阳(0.766 2). 图 1 表 3 参 14

关键词 茸鹿;品种(品系);RAPD;遗传变异

CLC Q75;S865.42

GENETIC DIVERSITY DETECTED BY RAPD IN FIVE VELVET – DEER BREEDS OR STRAINS OF CHINA

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Abstract RAPD analysis was used to estimate genetic diversity and relationship in 75 individuals which belong to five deer breeds or strains. 41 primers were used, among which 40 generated polymorphic loci. A total of 359 bands were scored, of which 259 (65.6%) were polymorphic. Shannon index was used to estimate the genetic diversity and the proportion of genetic variation within and between populations. The genetic distance among individuals was obtained by proportion of shared fragments. Dendrogram was generated by UPGMA and reflected the genetic relationship among the five deer breeds or strains. The results indicated that the genetic diversity of Shuangyang was the lowest in the five populations. About 54% variation was detected within populations and 46% among populations. The genetic diversity index of five breeds and strains was 1.124 9 in Xifeng, 1.051 7 in Changbaishan, 0.990 8 in Wulanba, 0.974 8 in Qingyuan and 0.766 2 in Shuangyang. Fig 1, Tab 3, Ref 14

Keywords deer; breed (strain); RAPD; genetic variation

CLC Q75;S865.42

DNA 分子水平上多态性检测技术是进行基因组研究的重要基础.随着 DNA 重组、限制酶消化、分子杂交、PCR 和核酸序列分析等分子生物学方法的建立,DNA 多态性检测技术也不断发展和完善,并在动植物的遗传

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结构和进化研究中逐步得到应用.由 Williams 等[1]建立起来的以 PCR 为基础的 DNA 多态性检测技术(RAPD),具有检测灵敏度高,操作简便、安全、快捷,只需微量 DNA 和对受试基因组无特定要求等特点.RAPD 正越来越广泛地应用在动植物的分类鉴定、连锁遗传分析、基因定位、寻找特定基因和群体遗传结构等研究领域之中.关于鹿类动物 RAPD 分析的研究,国外仅见 Comincini E.等[2]利用设计的 8 条引物对麂、马鹿、□鹿、狍和黑尾鹿五种鹿科动物系统进化关系进行 RAPD 分析的研究报道,而国内迄今未见这方面的报道.我国鹿类动物资源丰富,利用现代分子生物学技术对其进行分子生物学方面的研究已实属非常必要.本研究对我国的 5 个茸鹿品种(品系)进行了 RAPD 研究,旨在通过分析它们的分子群体遗传结构,了解其群体遗传变异和亲缘关系,为合理利用我国珍贵的茸鹿资源提供科学依据.

1 材料与方法

1.1 试验材料

实验动物样品为茸鹿品种(品系):双阳品种(以下简称 SY,双阳二鹿场)、长白山品系(CB,通化一鹿场)、西丰品种(XF,西丰育才种鹿场)、清原品系(QY,清原县参茸场)和乌兰坝品种(WL,赤峰市巴林左旗乌兰坝林场鹿场)核心群鹿只随机取样的血液样品,共计 75 头份,每个品种(品系)各 15 个个体样品.眠乃宁麻醉鹿只,颈静脉采血 5 ~ 10 mL,肝素抗凝,-20℃保存备用.

1.2 主要仪器和试剂

PCR 仪为 AmpGene DNA Thermal Cycler 4800 型,凝胶自动成像仪为 STRATGENE Eagle Eye Ⅱ型;*Tag* DNA 聚合酶及 10 × Buffer 购自中国农业大学农业生物技术国家重点实验室,10 bp 随机引物购自上海生工生物工程公司(Sangon),其它药品与试剂均为国产 AR 级或进口分装 AR 级.

1.3 茸鹿血液 DNA 的提取

参照卢圣栋[3] DNA 提取的方法,稍加改进,从鹿冻融血中提取基因组 DNA.

1.4 PCR 反应及 RAPD 产物分离

PCR 反应体积为 25 μL,其中 c(Tris - HCl) = 10 mmol/L (pH 8.3),c(KCL) = 50 mmol/L,c($MgCl_2$) = 2.0 mol/L,每种 dNTP c(dNTP) = 0.2 mmol/L,1 u *Taq* DNA 聚合酶,10 pmol 引物和 15 ng DNA 模板.反应混合液置于 0.5 mL Eppendorf 管中,用 20 μL 石蜡油覆盖.每个反应 45 个循环,每个循环包括 94℃变性 80 s,36℃退火 60 s,72℃延伸 120 s,首次循环先在 95℃变性 180 s,最后一次循环后 72℃延伸 600 s,并且每次 PCR 反应均置不含 DNA 模板的空白对照(CK_0).反应产物用 1.5% 琼脂糖凝胶电泳分离,溴化乙锭染色,于 STRATGENE Eagle Eye Ⅱ自动成像仪上观察电泳图谱,打印出电泳结果并计算扩增片段的大小.

1.5 电泳图谱的分析及数据统计处理

只统计清晰可辨、可重复的 RAPD 谱带.扩增结果全部输入计算机,用 FORTRAN 语言编制计算机程序对扩增结果进行统计分析.

1.5.1 遗传距离(Genetic distance) 利用 Nei 氏片段共享度来计算个体间的遗传距离[4],公式为 $D = 1 - 2N_{ab}/(N_a + N_b)$,其中 N_a 是 a 个体扩增的总带数,N_b 是 b 个体扩增的总带数,N_{xy}是 x 个体和 y 个体共同享有的带数.

1.5.2 遗传多样性指数(Genetic diversity index)即 Shannon 指数[5] 品种(品系)遗传多样性指数 $H_o = -\sum P_i \ln P_i$;总群体遗传多样性指数 $H_{sp} = -\sum P \ln P$;群体内遗传多样性指数 $H_{pop} = 1/n \sum H_o$ 式中,P_i 为一条扩增谱带在品种(品系)内的分布频率,P 为一条扩增谱带在总群体中的分布频率,n 为品种(品系)数.根据公式计算出遗传多样性在群体内和群体间的分布,H_{pop}/H_{sp}为遗传多样性在群体内的比例,而($H_{sp} - H_{pop}$)/H_{sp}则为遗传多样性在群体间所占的比例.

1.5.3 系统聚类图 根据遗传距离,采用 UPGMA(Unweighted pair group method with arithmetic mean)方法,在 PHLIP Neighbor - Joining/UPGMA method version 3.573 c 软件上构建反映个体间遗传关系的系统聚类图.

2　结果与分析

2.1　RAPD 扩增结果

在所采用的 41 个引物中，只有引物 OPK05 扩增的谱带在所有受试个体均为一条带的单型，其它 40 个引物则表现不同程度的多态(25.0% ~ 90.0%)。扩增片段的 Mr max = 3354 bp，Mr min = 191 bp；引物 OPK15 扩增片段数最多，为 18 条；41 个引物共检测到 395 条扩增片段，其中 259 条为多态，多态片段占总扩增片段的比例为 65.6%。多态片段在不同茸鹿品种(品系)中的分布也不相同，综合 40 个多态引物扩增的结果，XF、CB、WL 的多态频率(分别平均为 79.5%，76.8%，76.5%)明显高于 SY(65.6%)和 QY(60.2%)(见表 1)。

表 1　茸鹿品种(品系)41 个引物 RAPD 扩增结果

Table 1　The results of RAPD with 41 primers in five breeds or strains of velvet – deer

引物 Primers	引物序列 Sequence of primers	FS	NR	NP	PP					FP
					SY	CB	XF	QY	WL	
OPB01	GTTTCGCTCC	2082 – 281	10	9	5	7	7	8	7	90.0
OPB02	TGATCCCTGG	2071 – 227	7	6	5	5	6	5	4	85.7
OPB03	CATCCCCCTG	2486 – 291	12	7	4	4	6	6	5	58.3
OPB04	GGACTGGAGT	2417 – 226	11	8	6	8	8	1	6	72.7
OPB05	TGCGCCCTTC	2522 – 466	10	9	6	7	8	7	6	90.0
OPB06	TGCTCTGCCC	2692 – 546	12	9	6	8	7	6	5	75.0
OPB07	GGTGACGCAG	2307 – 517	13	9	7	6	4	2	3	69.2
OPB08	GTCCACACGG	2639 – 385	12	9	7	8	8	3	8	75.0
OPB09	TGGGGGACTC	2131 – 785	8	6	5	3	3	2	4	75.0
OPB10	CTGCTGGGAC	1034 – 233	9	3	0	3	1	1	3	33.3
OPB11	GTAGACCCGT	2798 – 251	11	8	4	6	7	6	8	72.7
OPB12	CCTTGACGCA	2799 – 191	13	8	3	7	7	6	7	61.5
OPB13	TTCCCCCGCT	665，376	2	1	1	0	0	0	0	50.0
OPB14	TCCGCTCTGG	2147 – 205	9	7	7	6	6	5	5	77.8
OPB15	GGAGGGTGTT	2410 – 391	13	5	2	3	5	3	4	38.5
OPB16	TTTGCCCGGA	2513 – 261	9	6	5	1	1	5	1	66.7
OPB17	AGGGAACGAG	3354 – 505	12	9	4	9	9	7	9	75.0
OPB18	CCACAGCAGT	2776 – 469	8	5	4	3	4	3	3	62.5
OPB19	ACCCCCGAAG	947 – 469	4	1	0	1	0	0	0	25.0
OPB20	GGACCCTTAC	3089 – 524	13	9	7	6	5	4	7	69.2
OPK01	CATTCGAGCC	2194 – 434	9	4	3	3	4	2	4	44.4
OPK02	GTCTCCGCAA	2788 – 308	11	8	5	5	4	2	5	72.7
OPK03	CCAGCTTAGG	2176 – 203	8	4	4	4	4	3	2	50.0
OPK04	CCGCCCAAAC	2174 – 491	13	8	6	6	6	5	7	61.5
OPK05	TCTGTCGAGG	1455	1	0	0	0	0	0	0	0
OPK06	CACCTTTCCC	2191 – 339	10	7	6	5	7	6	5	70.0
OPK07	AGCGAGCAAG	2811 – 209	9	7	3	7	7	4	7	77.8
OPK08	GAACACTGGG	2122 – 393	11	9	6	7	6	5	6	81.8
OPK09	CCCTACCGAC	2309 – 275	10	7	5	5	5	5	7	70.0
OPK10	GTGCAACGTG	2687 – 529	14	11	8	9	7	7	9	78.6
OPK11	AATGCCCCAG	2545 – 428	12	11	9	7	11	2	7	91.7
OPK12	TGGCCCTCAC	1986 – 259	8	4	3	3	4	2	4	50.0
OPK13	GGTTGTACCC	2262 – 330	10	4	1	4	4	3	5	40.0
OPK14	CCCGCTACAC	2814 – 377	10	8	5	5	7	6	7	80.0
OPK15	CTCCTGCCAA	2888 – 396	18	11	9	9	10	9	10	61.1
OPK16	GAGCGTCGAA	2830 – 837	4	3	3	2	2	3	2	75.0
OPK17	CCCAGCTGTG	1513 – 312	8	2	2	1	2	2	2	25.0
OPK18	CCTAGTCGAG	730，1980	2	1	0	1	0	0	0	50.0
OPK19	CACAGGCGGA	2043 – 315	11	7	5	6	7	5	7	63.6
OPK20	GTGTCGCGAG	2459 – 370	11	5	3	5	3	1	4	45.5
OPL05	AGGCAGGCAC	2523 – 635	7	4	3	4	4	4	4	57.1
总数 Total			395	259	177	199	206	156	198	
多态% Multiform %				65.6	68.3	76.8	79.5	60.2	76.5	

FS 扩增片段大小(bp)的范围；NR：RAPD 标记数；NP：多态数；PP：多态标记在品种(品系)中的分布；FP：多态频率%；SY：双阳品种；CB：长白山品系；XF：西丰品种；QY：清原品系；WL：乌兰坝品种

FS：Fragment size；NR：Number of RAPD；NP：Number of polymorphism；PP：Polymorphism within population；FP：Frequency of polymorphism；SY：Shuangyang；CB：Changbaishan；XF：Xifeng；QY：Qingyuan；WL：Wulanba

2.2 5个茸鹿品种(品系)的遗传变异

根据40个多态引物RAPD谱带在各品种(品系)内的分布,用Shannon指数(H)表达了5个品种(品系)的遗传多样性指数(见表2). 由表知,SY、QY群体内变异较低,而XF和CB的群体内变异最高,WL的群体内变异居中.

SY是1986年我国也是世界上首次育成的鹿类动物第一个培育品种,其原始群体是清咸丰年间(1833)捕捉的当地野生梅花鹿而形成的家养群体,并经过长期人工驯养,形成了具有一定特点的地方类型鹿群 – 双阳梅花鹿,特别经过近50年的人工选育提高而形成了双阳梅花鹿品种,从形成历史看其选育程度高且遗传背景较为单一. QY是辽宁省外贸为原清原县种畜场从新疆自治区伊犁哈萨克自治州特克斯县境内,引种111只天山马鹿,经过风土驯化、扩繁选育而形成的;其原始群体较小,遗传基础相对较窄,选育程度较高,遗传变异程度较低. XF其原始群体大,是由原始群体组建时间不同的西丰县三个鹿场联合育种而形成的,其遗传基础相对较丰富. 长白山品系在培育过程中,曾引进蛟河型梅花鹿和辉南县鹿场梅花鹿,遗传背景相对较为复杂. 本研究的结果表明,RAPD数据基本反映了这些客观情况.

研究群体遗传结构,不仅要度量群体遗传变异的高低,更重要的是估测遗传变异在群体内和群体间的分布,这对于了解遗传变异产生的机制,进而制定选种及利用措施都极为重要[6].

动植物品种间和品种内遗传变异丰富,而RAPD检测遗传变异的灵敏度高,所以是分析群体遗传结构的理想工具,从本研究对5个茸鹿品种(品系)75个个体的分析结果提示(见表2),遗传变异主要存在于品种内(占53.97%),品种间的遗传变异居次要地位(占46.05%). 对于大多数异交动植物,遗传变异则主要存在于群体内,而对于自交生物来讲,遗传变异则主要存在于群体间[7],本研究数据与群体遗传学的这一基本定律相符合.

2.3 5个茸鹿品种(品系)的遗传分化

表3是根据Nei氏[4]片段共享度计算得到的5个茸鹿品种(品系)75个个体间的遗传距离. 个体间遗传距离的范围在0.06 ~ 0.26之间,为了形象地表示5个茸鹿品种(品系)个体间的遗传关系,用UPGMA方法聚类构建了个体间的系统发育树状图(见图1).

从图1个体间聚类树状图可以看出,同一品种(品系)内的个体最先聚在一起,75个个体可以归结为5个明显区分的类群,分别对应于SY、CB、XF、WL和QY. 一般来说,同品种的个体所处的生态地理条件和人工选择的方向基本相同,群体来源也较一致,具有大致相同的形态外貌和生产性能,因而遗传基础也比较一致,由RAPD分析而产生的系统聚类图基本反映了这一情况. 在图中,代表XF的第一类群和代表CB的第二类群首先聚在一起,而后与代表SY的第三个类群聚在一起;代表WL的第四类群与代表QY的第5个类群又聚在一起,最后代表5个类群的所有个体聚成一类. 个体间的聚类图表明XF、CB、SY之间以及QY、WL之间的亲缘关系较近.

3 讨 论

3.1 RAPD是研究茸鹿群体遗传结构理想的DNA标记

本研究所用的41个引物已将75个个体区分开,没有发现任何2个个体拥有完全相同的RAPD标记,说明这一技术检测遗传变异具有较高的灵敏度. 同时依据遗传距离的聚类分析结果表明,同品种(品系)内的个体大都聚成一类,又说明该技术具有一定的稳定性. 虽然在试验过程也会出现一些难以分别的弱带,但可通过重复实验、增加引物数量或将这些弱带排除在数据分析之外以减少或排除这种误差. RAPD试验中,由于引物数原则上可任意增加,因此有可能找到任何一个个体的特异性RAPD标记;另外,RAPD直接检测DNA而消除了蛋白多态技术中的一些限制和缺陷,又比线粒体DNA RFLP分辨率高,却又不象传统的DNA指纹图谱那样消耗昂贵且操作烦琐. 因此,RAPD技术在寻找遗传标记方面具有巨大潜力.

3.2 RAPD分析能很好地反映茸鹿群体的遗传多样性

RAPD是在分子水平上对基因型进行分析,其结果不受环境条件和其它基因作用的影响,即无表型效应,

能够检测出在表型上毫无差别的个体间在基因型上存在的差异，以检测出单拷贝序列及重复序列 DNA 多态性差异，RAPD 在检测生物遗传多样性与群体遗传变异上的有效性已被以往的研究所证实[8]. 对 5 个茸鹿品种(品系)的 RAPD 分析反映出了茸鹿品种(品系)遗传资源的丰富性，它们的遗传变异约 46% 存在于品种(品系)

表 2　茸鹿品种(品系)遗传多样性指数(Ho)及遗传多样性在群体间与群体内的分布

Table 2　The genetic diversity index(H_o) of five breeds or strains of velvet – deer and partition of genetic diversity within and among populations

引物 Primers	H_o					H_{sp}	H_{pop}	H_{pop}/H_{sp}	$(H_{sp}-H_{pop})/H_{sp}$
	SY	CB	XF	QY	WB				
OPB01	0.6932	1.4714	2.0467	1.6872	2.4262	2.7250	1.6649	0.6110	0.3890
OPB02	1.0409	0.9090	1.1684	1.6022	0.3466	1.3585	1.0134	0.7460	0.2540
OPB03	0.0000	1.4741	1.0498	0.9092	1.0398	1.9772	0.8946	0.4525	0.5475
OPB04	1.3864	0.5624	0.6913	0.3467	1.2555	2.2194	0.8485	0.3823	0.6177
OPB05	1.3868	1.2655	1.2255	0.5642	0.3477	2.0055	0.9579	0.4777	0.5224
OPB06	1.3894	1.0399	0.8672	1.0400	0.3467	2.3736	0.9366	0.3946	0.6054
OPB07	1.5568	2.0796	0.9354	0.3866	0.5824	2.2373	1.1082	0.4953	0.5047
OPB08	2.1686	1.7380	1.3891	0.3486	2.3884	2.8062	1.6065	0.5725	0.4275
OPB09	2.1646	0.5628	0.6973	0.3461	0.9096	1.4403	0.9361	0.6499	0.3501
OPB10	0	1.0398	0.3659	0.3496	0.9190	1.0005	0.5349	0.5346	0.4654
OPB11	1.0399	1.3864	1.9289	1.9488	2.4596	2.6896	1.7527	0.6517	0.3584
OPB12	0.0000	1.9030	1.5207	1.6032	1.1288	2.4298	1.2311	0.5067	0.4933
OPB13	0.3867	0	0	0	0	0.2180	0.0773	0.3548	0.6454
OPB14	1.4734	1.6122	1.5688	1.1348	1.5563	2.1851	1.4691	0.6723	0.3277
OPB15	0.0000	0.5658	1.0708	0.7787	0.9904	1.1836	0.6811	0.5755	0.4246
OPB16	0.7782	0.0000	0.0000	1.4256	0.0000	1.0057	0.4408	0.4383	0.5617
OPB17	0.9096	2.4664	2.5883	1.7380	0.6972	3.0281	1.6799	0.5548	0.4452
OPB18	0.6941	0.5634	0.7699	0.3476	0.5681	1.5750	0.5886	0.3737	0.6263
OPB19	0	0.3479	0	0	0	0.2181	0.0696	0.3192	0.6809
OPB20	0.3461	1.3496	1.2902	1.7338	0.7782	2.9901	1.0996	0.3677	0.6323
OPK01	0.3586	0.3566	0.6753	0.9940	0.3476	0.9839	0.5464	0.5554	0.4447
OPK02	0.2158	1.2582	1.0254	0.3496	0.7782	1.6070	0.7254	0.4514	0.5480
OPK03	0.3469	0.5624	0.9161	0.3466	0.2258	1.1084	0.4796	0.4327	0.5673
OPK04	0.0000	0.5644	1.7697	1.4785	1.2948	1.9912	1.0215	0.5130	0.4869
OPK06	0.0000	1.3416	2.0245	1.7387	0.6932	2.1343	1.1596	0.5433	0.4567
OPK07	0.7782	1.8180	1.7539	0.9191	1.9488	2.0853	1.4436	0.6923	0.3077
OPK08	0.3437	0.9200	0.6478	0.6932	0.9213	1.1889	0.7052	0.5932	0.4068
OPK09	1.3858	1.1248	1.1230	1.3864	1.9508	2.0898	1.3942	0.6671	0.3328
OPK10	1.7333	1.0398	2.0617	2.5112	1.4714	3.2021	1.7635	0.5507	0.4493
OPK11	2.7728	1.2486	3.1050	0.5639	1.6022	2.8947	1.8585	0.6420	0.3579
OPK12	0.0000	0.5623	0.9653	0.9207	0.4743	1.2591	0.5843	0.4641	0.5359
OPK13	0.0000	0.5633	1.0381	0.0000	1.1251	1.1700	0.5453	0.4661	0.5339
OPK14	0.0000	0.6479	2.0905	1.6032	0.7782	2.2897	1.0240	0.4472	0.5528
OPK15	1.7350	1.7722	3.6666	3.1194	2.6420	3.9314	2.5870	0.6581	0.3419
OPK16	0.6952	0.5664	0.7125	0.6937	0.6938	1.0317	0.6723	0.6517	0.3483
OPK17	0.0000	0.2158	0.3012	1.1298	0.3106	0.5174	0.3915	0.7566	0.2433
OPK18	0	0.3469	0	0	0	0.2182	0.0694	0.3182	0.6819
OPK19	1.3868	1.9488	1.7243	1.1299	1.8181	2.3318	1.6016	0.6868	0.3131
OPK20	0.6936	1.7343	0.9492	0.3444	1.0389	1.4523	0.9521	0.6556	0.3444
OPL05	0.7883	1.1398	0.8717	0.7782	0.7782	1.2243	0.8712	0.7116	0.2884
$\overline{X}$	0.7662	1.0517	1.1249	0.9748	0.9908	1.8095	0.9997	0.5397	0.4605

表3 由40个多态引物扩增得到的5个茸鹿品种(品系)75个个体的遗传距离

Table 3 The genetic distance of 75 individuals belonging to 5 breeds or stains of velvet – deer calculated from 40 polymorphic primers

SY02 .1411
SY03 .1637 .1148
SY04 .1746 .1428 .1275
SY05 .1626 .1123 .1016 .0456
SY06 .1102 .1115 .0858 .1094 .1324
SY07 .1230 .0590 .1053 .1064 .0988 .1285
SY08 .0861 .1230 .1082 .1708 .1785 .1434 .1554
SY09 .1137 .1111 .1263 .1204 .1281 .1119 .1204 .1196
SY10 .1354 .1089 .0871 .1106 .0954 .1329 .1068 .1059 .1593
SY11 .1155 .0822 .0983 .1030 .1068 .0751 .0992 .1213 .1362 .1455
SY12 .1164 .0982 .1028 .1076 .0961 .1029 .0730 .1495 .1605 .1389 .1081
SY13 .1224 .1232 .1233 .1367 .1367 .1282 .1171 .1203 .1434 .1058 .1372 .1343
SY14 .1066 .1042 .1198 .1059 .1059 .0933 .1136 .1167 .1124 .1257 .1025 .1150 .1564
SY15 .1371 .1068 .1000 .1314 .1238 .1153 .1047 .1269 .1149 .1281 .1128 .1136 .1780 .1621
CB01 .2048 .1867 .1660 .1844 .1728 .2000 .1922 .1843 .1914 .1812 .1734 .1984 .1856 .2007 .1906
CB02 .1944 .1771 .1571 .1825 .1749 .1900 .1863 .1708 .1892 .1679 .1717 .1884 .1835 .1830 .1733 .0873
CB03 .1624 .1954 .1715 .1932 .1894 .1704 .2007 .1515 .1811 .1902 .1600 .1992 .1791 .1825 .1879 .1685 .1557
CB04 .1968 .1755 .1777 .2114 .2076 .1807 .2000 .1846 .2030 .1969 .1701 .1907 .1859 .2007 .1984 .1400 .1580 .1654
CB05 .1921 .1789 .1773 .2180 .2030 .1840 .1917 .1878 .2098 .1924 .1811 .1939 .1891 .1923 .1977 .1247 .1240 .1465 .0659
CB06 .1764 .1902 .1627 .1917 .1917 .1916 .1992 .1499 .1795 .1924 .1584 .1863 .1776 .1923 .1864 .1055 .1278 .0909 .1148 .1561
CB07 .1943 .1769 .1529 .1938 .1823 .1705 .1861 .1782 .1814 .1753 .1714 .1961 .1834 .1828 .1846 .0941 .0518 .1363 .1384 .1309 .1574
CB08 .1902 .1883 .1826 .1782 .1744 .1937 .2015 .1780 .1968 .1750 .1712 .2000 .1832 .2102 .1844 .0693 .0930 .1204 .1572 .1685 .1302 .1232
CB09 .1823 .1653 .1567 .1708 .1669 .1782 .1785 .1511 .1698 .1753 .1483 .1844 .1637 .1867 .1769 .0980 .0978 .1136 .1423 .1688 .0967 .0968 .0919
CB10 .1896 .2068 .1895 .1969 .1931 .1969 .2045 .1853 .1961 .2053 .1861 .2030 .1905 .2054 .1915 .1132 .0975 .1169 .1341 .1153 .1342 .1042 .1033 .1351
CB11 .1834 .1704 .1654 .2060 .1984 .1793 .1909 .1679 .1977 .1802 .1612 .1892 .1922 .1800 .1818 .1119 .1077 .1156 .0909 .0953 .0990 .1221 .1252 .1450 .1711
CB12 .2007 .1793 .1592 .1885 .1771 .1846 .1923 .1846 .1954 .1663 .1778 .1907 .1780 .1891 .1984 .0778 .0857 .1390 .1030 .0847 .1111 .0884 .1184 .1500 .1455 .1022
CB13 .1868 .1889 .1717 .1748 .1787 .1785 .1905 .1746 .1818 .1873 .1597 .1928 .1757 .1832 .1850 .1244 .1198 .1356 .1141 .1145 .1300 .1190 .1382 .1269 .1264 .1367 .1102
CB14 .2063 .2000 .1866 .2129 .2053 .2092 .2129 .1785 .2045 .1984 .2022 .2076 .2070 .2100 .2038 .0951 .1026 .1069 .1428 .1390 .1090 .1132 .9302 .1055 .1128 .1153 .1200 .1787
CB15 .1928 .1908 .1666 .2000 .1923 .1884 .1961 .1846 .1992 .1892 .1701 .1946 .1780 .1969 .1946 .1050 .1200 .1353 .0954 .1186 .1035 .1269 .1145 .1307 .1187 .1060 .1068 .1496 .1542
XF01 .1767 .1992 .2046 .2047 .2087 .1967 .2087 .1767 .2000 .1936 .1976 .1871 .2024 .2016 .2071 .1991 .2047 .1960 .1912 .1866 .1866 .2048 .2170 .1927 .1960 .1936 .1952 .2181 .2246 .1912
XF02 .1975 .1835 .1893 .1968 .1929 .1850 .1929 .2047 .1843 .1937 .1819 .2031 .2144 .1976 .1875 .1713 .1851 .1769 .1718 .1676 .1714 .1889 .1809 .1692 .1843 .1744 .1679 .1854 .2007 .1601 .1224
XF03 .1680 .1709 .1961 .2156 .2039 .2039 .1803 .1643 .1794 .1929 .1850 .2023 .1975 .1848 .1866 .1623 .1764 .1760 .1984 .1744 .1744 .1920 .1760 .1801 .1834 .1656 .1787 .1764 .1921 .1905 .1375 .1227
XF04 .1695 .1835 .1672 .1887 .1813 .1698 .1887 .1660 .1729 .1894 .1594 .1795 .1900 .1704 .1947 .1717 .1700 .1623 .1647 .1534 .1608 .1735 .1771 .1660 .1804 .1561 .1573 .1776 .1850 .1573 .1679 .1494 .1599
XF05 .1560 .1785 .1582 .1724 .1570 .1721 .1724 .1605 .1753 .1730 .1615 .1511 .1811 .1805 .1554 .1663 .1570 .1644 .1976 .1818 .1704 .1644 .1757 .1605 .1714 .1771 .1708 .1722 .1877 .1708 .1743 .1827 .1739 .1337
XF06 .1905 .1471 .1318 .1713 .1563 .1634 .1525 .1673 .1590 .1606 .1531 .1733 .1644 .1717 .1660 .1807 .1713 .1561 .1773 .1843 .1508 .1749 .1823 .1596 .1856 .1647 .1698 .1712 .1826 .1660 .1771 .1776 .1650 .1444 .1385
XF07 .1722 .1596 .1365 .1612 .1574 .1570 .1537 .1570 .1603 .1504 .1466 .1669 .1890 .1653 .1520 .1705 .1764 .1685 .1901 .1782 .1669 .1762 .1837 .1724 .1870 .1735 .1673 .1882 .1954 .1673 .1309 .1284 .1428 .1231 .1166 .0789
XF08 .1714 .1819 .1878 .1953 .1835 .1952 .1875 .1676 .1866 .1921 .1686 .1936 .1927 .1801 .1898 .1696 .1640 .1676 .1859 .1660 .1698 .1834 .1792 .1637 .1748 .1689 .1702 .1676 .1914 .1702 .1411 .1462 .1451 .1324 .1102 .1295 .1423
XF09 .1914 .1819 .1730 .1760 .1685 .1947 .1722 .1947 .1826 .1842 .1804 .1856 .1961 .1840 .1969 .1854 .1872 .1866 .1932 .1851 .1703 .1984 .1984 .1871 .2052 .1769 .1744 .1992 .2022 .1857 .1506 .1401 .1467 .0939 .1245 .1391 .1289 .1692
XF10 .1693 .1721 .1782 .2162 .2007 .1890 .1969 .1539 .1805 .1937 .1744 .1835 .1825 .1898 .1876 .1518 .1544 .1542 .1566 .1603 .1412 .1656 .1574 .1423 .1611 .1401 .1644 .1736 .1621 .1605 .1313 .1247 .0836 .1195 .1400 .1395 .1522 .1349 .1634
XF11 .1814 .1682 .1632 .1891 .1853 .1656 .1853 .1734 .1728 .1860 .1589 .1718 .1865 .1937 .1605 .1715 .1930 .1771 .1644 .1717 .1564 .1929 .1929 .1812 .1805 .1746 .1682 .1736 .1969 .1721 .1232 .0930 .1274 .1423 .1595 .1395 .1329 .1746 .1634 .1254

XF12 .1868 .1968 .2061 .2180 .2062 .1944 .2023 .1984 .2055 .2031 .2071 .1968 .2000 .2031 .2204 .2088 .2062 .1937 .2125 .1883 .2116 .2063 .2104 .1944 .2173 .2031 .1929 .2154 .2337 .1929 .1028 .1250 .1237 .1428 .1683 .1673 .1529 .1717 .1605 .1616 .1656

XF13 .1891 .2191 .2123 .2246 .2167 .2048 .2087 .2128 .2200 .2015 .2175 .2032 .2188 .2056 .2270 .1991 .1968 .1803 .2270 .2023 .1866 .2168 .2048 .2088 .2120 .1818 .1872 .2057 .2007 .1992 .1791 .1755 .1540 .1601 .1462 .1614 .1587 .1411 .1389 .1515 .2040 .1851

XF14 .1755 .1628 .1617 .1795 .1720 .1832 .1569 .1641 .1673 .1726 .1612 .1816 .1961 .1762 .1666 .1737 .1720 .1753 .1780 .1738 .1738 .1717 .1830 .1564 .1749 .1766 .1818 .1835 .1909 .1818 .1225 .1201 .1189 .1226 .1352 .1385 .1207 .1456 .1098 .1247 .1209 .1445 .2173

XF15 .1757 .1899 .1691 .1914 .1953 .1757 .2030 .1640 .1789 .1883 .1728 .1741 .1968 .1843 .1821 .1739 .1798 .1793 .1666 .1739 .1510 .1914 .1834 .1718 .1828 .1769 .1744 .1800 .1992 .1627 .1133 .1349 .1257 .1178 .1461 .1341 .1274 .1451 .1504 .1041 .1041 .1400 .2064 .1500

WL01 .2278 .2282 .2211 .2137 .2096 .2301 .2177 .2342 .2373 .2186 .2145 .2285 .2074 .2188 .2242 .1835 .2016 .2286 .2363 .2270 .2270 .2097 .1975 .2097 .2170 .2184 .2040 .2108 .2137 .2040 .2473 .1925 .2000 .2237 .1991 .2135 .2152 .2033 .2222 .1967 .2213 .2442 .2262 .2144 .2238

WL02 .2424 .2256 .2344 .2603 .2479 .2609 .2231 .2400 .2390 .2406 .2448 .2510 .2255 .2368 .2505 .2135 .2148 .2464 .2587 .2571 .2367 .2233 .2194 .2108 .2349 .2361 .2422 .2419 .2314 .2339 .2537 .2271 .2094 .2535 .2375 .2433 .2453 .2255 .2520 .2268 .2352 .2376 .2494 .2402 .2463 .1497

WL03 .2435 .2269 .2514 .2693 .2571 .2577 .2326 .2453 .2402 .2500 .2500 .2644 .2268 .2463 .2638 .2233 .2163 .2474 .2515 .2298 .2500 .2288 .2250 .2288 .2443 .2292 .2229 .2389 .2489 .2351 .2419 .2201 .2109 .2464 .2510 .2565 .2505 .2268 .2489 .2157 .2406 .2389 .2548 .2292 .2557 .1521 .1205

WL04 .2400 .2360 .2441 .2654 .2614 .2459 .2375 .2500 .2409 .2424 .2505 .2484 .2320 .2591 .2360 .2163 .2095 .2283 .2560 .2386 .2268 .2217 .2138 .2258 .2249 .2341 .2200 .2396 .2175 .2240 .2594 .2213 .2247 .2392 .2434 .2371 .2390 .2443 .2455 .2210 .2373 .2561 .2426 .2460 .2439 .1422 .1241 .1483

WL05 .2410 .2249 .2295 .2585 .2505 .2469 .2224 .2307 .2419 .2313 .2434 .2454 .2206 .2439 .2369 .2172 .2144 .2252 .2530 .2316 .2396 .2226 .2106 .2226 .2258 .2390 .2168 .2282 .2224 .2249 .2394 .2181 .2049 .2362 .2323 .2182 .2280 .2329 .2465 .2016 .2138 .2365 .2478 .2191 .2285 .1130 .1203 .1144 .0548

WL06 .2360 .2361 .2524 .2540 .2418 .2546 .2336 .2505 .2453 .2427 .2510 .2531 .2236 .2474 .2566 .2033 .2049 .2565 .2525 .2307 .2388 .2173 .2217 .2215 .2371 .2301 .2197 .2271 .2459 .2279 .2559 .2126 .2118 .2474 .2396 .2616 .2515 .2278 .2459 .2166 .2458 .2441 .2430 .2301 .2567 .1179 .0941 .0707 .1231 .1323

WL07 .2415 .2332 .2416 .2469 .2510 .2515 .2348 .2351 .2382 .2276 .2439 .2581 .2166 .2402 .2494 .2132 .2307 .2375 .2535 .2520 .2320 .2433 .2107 .2229 .2382 .2273 .2373 .2620 .2267 .2089 .2399 .2141 .2217 .2445 .2408 .2384 .2242 .2291 .2509 .2222 .2345 .2452 .2271 .2515 .2453 .1250 .0707 .1310 .1044 .1092 .1491

WL08 .2435 .2269 .2356 .2489 .2326 .2536 .2244 .2577 .2402 .2418 .2336 .2438 .2226 .2380 .2392 .2025 .2040 .2354 .2433 .2338 .2379 .2123 .2125 .2206 .2238 .2210 .2147 .2135 .2244 .2269 .2676 .2117 .2067 .2464 .2263 .2363 .2464 .2058 .2369 .2157 .2406 .2642 .2462 .2373 .2474 .0826 .1205 .1189 .1397 .1619 .1238 .1659

WL09 .2434 .2349 .2474 .2489 .2365 .2578 .2406 .2452 .2359 .2333 .2541 .2563 .2264 .2547 .2432 .1974 .2033 .2351 .2515 .2336 .2336 .2117 .2118 .2117 .2233 .2206 .2266 .2086 .2282 .2266 .2549 .2068 .1931 .2382 .2343 .2525 .2505 .2179 .2367 .2151 .2405 .2301 .2374 .2371 .2389 .1106 .1000 .1165 .1247 .1516 .0900 .1288 .0852

WL10 .2591 .2295 .2460 .2556 .2515 .2520 .2351 .2561 .2469 .2443 .2443 .2629 .2294 .2489 .2540 .2217 .2188 .2379 .2540 .2404 .2444 .2272 .2192 .2272 .2386 .2317 .2254 .2457 .2392 .2254 .2489 .2058 .2135 .2530 .2453 .2348 .2326 .2294 .2515 .2266 .2224 .2415 .2532 .2195 .2625 .1241 .1006 .0816 .0991 .0909 .1130 .0984 .1125 .1235

WL11 .2327 .2168 .2256 .2424 .2304 .2469 .2104 .2348 .2338 .2233 .2233 .2454 .2247 .2235 .2329 .2049 .2104 .2411 .2289 .2198 .2356 .2145 .2188 .2186 .2338 .2231 .2048 .2240 .2224 .2208 .2478 .2098 .2049 .2362 .2282 .2222 .2320 .2247 .2347 .1975 .2179 .2489 .2394 .2231 .2326 .1087 .1028 .0971 .1054 .0805 .0889 .1134 .1360 .1516 .1385

WL12 .2320 .2363 .2407 .2540 .2500 .2423 .2338 .2382 .2454 .2469 .2429 .2367 .2199 .2392 .2444 .2123 .2096 .2405 .2606 .2470 .2390 .2301 .2057 .2179 .2292 .2384 .2282 .2484 .2258 .2242 .2558 .2339 .2250 .2316 .2276 .2455 .2434 .2323 .2460 .2090 .2459 .2567 .2431 .2464 .2402 .1030 .1013 .1217 .0785 .0660 .1179 .0905 .1304 .1637 .1285 .1044

WL13 .2370 .2494 .2375 .2592 .2592 .2474 .2386 .2474 .2422 .2479 .2396 .2541 .2245 .2526 .2536 .2210 .2222 .2454 .2536 .2520 .2398 .2349 .2100 .2307 .2380 .2351 .2329 .2366 .2510 .2123 .2527 .2093 .2255 .2444 .2448 .2464 .2320 .2288 .2469 .2301 .2384 .2537 .2527 .2433 .2494 .1140 .1036 .1111 .1149 .1111 .1071 .1189 .1066 .1312 .1002 .1198 .1052

WL14 .2703 .2443 .2604 .2581 .2540 .2712 .2540 .2670 .2577 .2510 .2633 .2697 .2362 .2598 .2566 .2117 .2213 .2484 .2648 .2469 .2469 .2256 .2343 .2298 .2494 .2382 .2197 .2399 .2336 .2402 .2516 .2252 .2161 .2595 .2396 .2494 .2638 .2447 .2419 .2291 .2416 .2399 .2430 .2423 .2609 .0960 .1121 .1283 .1144 .1193 .1155 .1096 .1061 .0900 .1086 .1236 .1222 .1741

WL15 .2393 .2229 .2594 .2571 .2448 .2536 .2285 .2412 .2443 .2459 .2500 .2520 .2310 .2505 .2433 .2108 .2163 .2273 .2515 .2379 .2338 .2247 .2041 .2164 .2156 .2332 .2310 .2304 .2163 .2351 .2419 .2201 .1983 .2464 .2304 .2404 .2342 .2226 .2570 .2033 .2282 .2389 .2419 .2332 .2474 .1478 .1160 .1057 .1225 .1231 .0884 .1441 .1365 .1390 .1258 .1058 .1130 .1377 .1814

QY01 .2240 .2015 .2104 .2107 .2068 .2224 .2030 .2185 .2292 .2076 .2038 .2093 .2007 .2194 .2360 .2485 .2452 .2438 .2514 .2613 .2537 .2456 .2421 .2301 .2524 .2419 .2514 .2475 .2567 .2476 .2304 .2377 .2490 .2316 .2277 .2220 .2313 .2322 .2301 .2217 .2295 .2396 .2545 .2190 .2358 .2195 .1791 .2057 .1951 .1838 .1983 .1795 .2304 .2133 .2041 .1797 .1991 .2074 .2107 .2181

QY02 .2222 .2000 .2125 .2091 .2205 .2053 .2015 .2168 .2160 .2213 .2099 .2038 .2148 .2023 .2266 .2427 .2395 .2195 .2380 .2481 .2218 .2399 .2403 .2245 .2543 .2287 .2342 .2298 .2395 .2190 .2127 .2241 .2470 .2299 .2337 .2165 .2144 .2226 .2284 .2355 .2239 .2298 .2485 .2136 .2147 .2217 .2148 .2244 .2295 .2104 .2295 .2105 .2408 .2282 .2188 .2184 .2298 .2304 .2295 .2408 .1302

QY03 .2363 .2093 .2180 .2263 .2301 .2343 .2108 .2187 .2412 .2194 .2194 .2172 .2246 .2235 .2325 .2332 .2417 .2442 .2558 .2543 .2428 .2500 .2426 .2382 .2490 .2384 .2441 .2600 .2340 .2480 .2145 .2500 .2335 .2357 .2319 .2260 .2277 .2206 .2380 .2180 .2416 .2480

.2388 .2192 .2322 .1991 .1789 .2058 .1991 .1836 .2108 .1711 .2182 .2093 .1958 .1836 .1991 .2201 .1899 .2224 .0955 .1025
QY04 .2440 .2284 .2029 .2145 .2337 .2147 .2260 .2108 .2369 .2269 .2153 .2209 .2362 .2194 .2245 .2289 .2298 .2287 .2437 .2462 .2234 .2301 .2460 .2301 .2485 .2304 .2207 .2316 .2298 .2322 .2104 .2377 .2411 .2241 .2123 .2258 .2122 .2204 .2301 .2256 .2295 .2356 .2424 .2228 .2241 .2073 .2000 .2222 .2233 .1959 .2190 .1877 .2469 .2343 .2123 .2040 .2113 .2282 .2107 .2263 .1119 .0842 .0838
QY05 .2380 .2228 .2014 .2243 .2281 .2207 .2243 .2092 .2390 .2213 .2175 .2192 .2421 .2177 .2266 .2271 .2433 .2345 .2495 .2481 .2293 .2514 .2403 .2360 .2543 .2287 .2342 .2534 .2281 .2342 .2087 .2397 .2352 .2299 .2222 .2241 .2182 .2265 .2247 .2162 .2316 .2455 .2405 .2249 .2263 .2137 .1983 .2163 .2175 .1943 .2295 .1821 .2408 .2323 .2147 .1983 .2056 .2263 .2131 .2326 .0996 .0950 .0715 .0344
QY06 .2255 .1915 .2118 .2084 .2237 .2200 .1931 .2046 .2153 .2168 .2053 .2030 .2023 .2093 .2260 .2382 .2351 .2452 .2528 .2589 .2362 .2432 .2475 .2277 .2538 .2357 .2490 .2569 .2390 .2337 .2120 .2392 .2426 .2218 .2177 .2196 .2137 .2102 .2391 .2194 .2310 .2332 .2440 .2129 .2217 .2129 .1891 .2156 .2088 .1935 .2123 .1812 .2361 .2233 .2057 .1975 .2089 .2256 .2123 .2238 .0944 .0783 .0389 .0751 .0936
QY07 .2289 .2292 .2112 .2230 .2269 .2233 .2192 .2194 .2417 .2239 .2200 .2178 .2252 .2241 .2292 .2337 .2346 .2144 .2408 .2471 .2319 .2271 .2313 .2233 .2417 .2237 .2331 .2167 .2346 .2292 .2193 .2347 .2380 .2287 .2286 .2228 .2245 .2134 .2348 .2226 .2304 .2445 .2474 .2122 .2328 .2122 .1882 .2148 .2080 .1845 .2157 .1885 .2272 .2142 .2049 .1926 .2081 .2083 .2116 .2231 .0891 .0576 .0802 .0581 .0692 .0986
QY08 .2363 .1976 .2142 .2030 .2108 .2109 .2069 .2304 .2295 .2077 .2077 .2093 .2047 .2117 .2403 .2529 .2417 .2404 .2441 .2543 .2428 .2421 .2504 .2343 .2529 .2500 .2325 .2400 .2572 .2480 .2307 .2380 .2574 .2433 .2397 .2298 .2316 .2485 .2304 .2455 .2377 .2320 .2550 .2269 .2283 .2114 .1957 .2141 .2154 .2040 .2025 .1958 .2349 .2135 .2083 .2000 .2197 .2243 .2066 .2307 .0565 .0870 .0944 .1111 .1334 .0972 .1154
QY09 .2375 .1992 .1933 .1931 .2007 .2084 .1931 .2239 .2269 .2015 .2015 .1992 .2102 .2015 .2298 .2382 .2237 .2264 .2452 .2514 .2249 .2277 .2436 .2200 .2500 .2281 .2260 .2292 .2351 .2413 .2120 .2235 .2426 .2218 .2369 .2159 .2175 .2298 .2165 .2233 .2233 .2292 .2360 .2129 .2256 .2210 .1975 .2073 .2168 .2016 .2082 .1975 .2361 .2150 .2098 .1935 .2292 .2256 .2123 .2238 .0789 .0745 .0817 .0905 .1128 .0807 .0676 .0622
QY10 .2304 .2038 .2089 .2284 .2245 .2209 .2207 .2054 .2355 .2215 .2061 .2155 .2110 .2256 .2269 .2431 .2437 .2386 .2461 .2561 .2371 .2480 .2407 .2248 .2664 .2404 .2423 .2380 .2514 .2423 .2208 .2362 .2475 .2377 .2147 .2357 .2260 .2307 .2325 .2280 .2241 .2380 .2650 .2175 .2226 .2138 .1858 .2000 .2096 .1862 .2049 .1820 .2412 .2201 .2066 .1862 .1975 .2182 .2132 .2164 .0754 .0710 .0820 .0754 .0671 .0772 .0757 .0859 .1042
QY11 .2286 .2175 .2111 .2152 .2342 .2115 .2152 .2153 .2260 .2275 .2122 .2138 .2289 .2162 .2290 .2256 .2342 .2255 .2328 .2391 .2241 .2307 .2388 .2346 .2298 .2234 .2290 .2362 .2266 .2213 .2191 .2460 .2416 .2247 .2245 .2037 .2167 .2133 .2345 .2185 .2301 .2519 .2390 .2159 .2209 .2080 .2008 .2310 .2120 .1967 .2238 .1967 .2310 .2266 .2090 .2088 .2161 .2247 .2114 .2392 .0978 .0857 .0542 .0556 .0742 .0613 .0751 .0891 .0957 .1115
QY12 .2307 .2194 .2316 .2286 .2364 .2367 .2131 .2211 .2358 .2295 .2295 .2235 .2310 .2220 .2349 .2475 .2519 .2466 .2699 .2681 .2567 .2563 .2490 .2446 .2553 .2524 .2582 .2665 .2441 .2427 .2170 .2485 .2440 .2380 .2304 .2360 .2263 .2231 .2442 .2401 .2480 .2344 .2494 .2292 .2386 .2057 .1856 .2166 .2097 .1901 .2217 .1735 .2291 .2245 .2066 .1983 .1975 .2184 .2050 .2291 .0898 .0813 .0611 .0507 .0426 .0643 .0666 .1124 .1189 .0802 .0757
QY13 .2144 .2000 .2089 .2168 .2245 .2131 .2092 .1976 .2239 .2138 .1984 .2116 .2031 .2178 .2153 .2509 .2514 .2272 .2500 .2523 .2333 .2519 .2524 .2325 .2586 .2366 .2500 .2420 .2552 .2307 .2088 .2362 .2435 .2301 .2301 .2205 .2183 .2228 .2362 .2163 .2280 .2460 .2530 .2099 .2265 .2179 .2025 .2123 .2298 .2105 .2256 .1860 .2371 .2285 .2066 .2145 .2219 .2224 .2215 .2412 .0986 .0748 .0820 .0831 .0825 .0810 .0834 .0937 .1042 .0813 .0846 .0763
QY14 .2365 .2099 .2037 .2114 .2190 .2153 .2114 .2153 .2298 .2122 .2122 .2138 .2172 .2123 .2251 .2295 .2342 .2255 .2366 .2467 .2128 .2423 .2388 .2230 .2490 .2234 .2213 .2283 .2228 .2404 .2151 .2304 .2220 .2322 .2245 .2150 .2167 .2172 .2307 .2147 .2185 .2362 .2231 .2159 .2325 .2000 .1925 .2147 .2120 .1927 .1991 .1886 .2269 .2182 .2049 .1847 .2121 .2247 .1991 .2106 .0786 .0895 .0736 .0556 .0704 .0842 .0597 .0813 .0727 .0769 .0725 .0834 .1038
QY15 .2398 .2163 .2173 .2101 .2178 .2298 .2140 .2259 .2407 .2187 .2187 .2125 .2240 .2189 .2358 .2286 .2334 .2514 .2436 .2461 .2576 .2298 .2380 .2337 .2524 .2340 .2319 .2394 .2490 .2358 .2097 .2295 .2409 .2351 .2313 .2331 .2271 .2320 .2260 .2252 .2252 .2354 .2586 .2185 .2158 .2107 .1949 .2092 .2065 .1827 .2142 .1867 .2343 .2085 .2117 .1868 .2066 .2109 .2058 .2259 .0823 .0856 .0653 .0745 .0817 .0645 .0748 .0851 .0724 .0805 .0706.0753 .1080 .1111

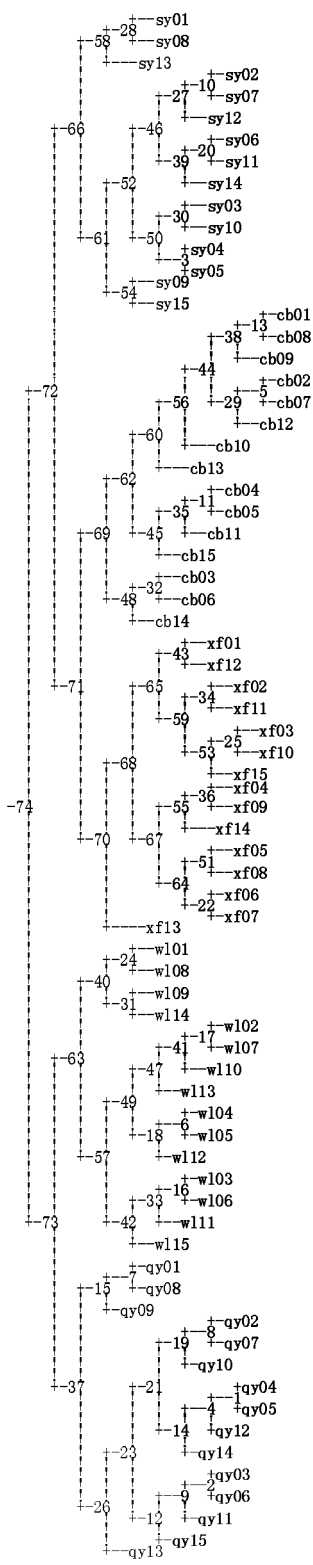
注:行数据对应的个体编号依次为 SY01, SY02, SY03, ..., SY15, CB01, CB02, CB03, ..., CB15, XF01, XF02, XF03, ..., XF15, WL01, WL02, ..., WL15, QY01, QY02, ..., QY13, QY14.

Notes: Numerals lined in row, corresponding the individual number in proper order, were SY01, SY02, SY03, ..., SY15, CB01, CB02, CB03, ..., CB15, XF01, XF02, XF03, ..., XF15, WL01, WL02, ..., WL15, QY01, QY02, ..., QY13, QY14.

间,表明各品种(品系)遗传分化已较明显且具丰富的遗传多样性.通过 RAPD 方法检测到西丰品种、长白山品系的遗传多样性指数即品种(品系)内遗传变异高于其它 3 个品种(品系),较高的遗传多样性表明它们具有较强的环境适应能力和进化潜力,因而可供育种的遗传素材也较丰富,而双阳品种的种内遗传多样性指数最低,表明其群体遗传变异低,这可能与其具有较高的选育程度直接有关. RAPD 分析所得到的茸鹿群体遗传多样性的结果基本上反映了原始群体起源、品种形成的背景与实际情况.

3.3 RPAD 分析反映了茸鹿群体的遗传分化

系统进化的研究已经表明,梅花鹿与马鹿是两个近缘种,马鹿是从梅花鹿群体中分化出来的且马鹿是在



Between	And	Length	Between	And	Length
74	72	0.02378	53	XF15	0.05747
72	66	0.02501	68	67	0.00581
66	58	0.00655	67	55	0.01002
58	28	0.01761	55	36	0.01118
28	SY01	0.04309	36	XF04	0.04696
28	SY08	0.04309	36	XF09	0.04696
58	SY13	0.06069	55	XF14	0.05814
66	61	0.00509	67	64	0.00228
61	52	0.00560	64	51	0.01076
52	46	0.00290	51	XF05	0.05512
46	27	0.01081	51	XF08	0.05512
27	10	0.01331	64	22	0.02641
10	SY02	0.02952	22	XF06	0.03947
10	SY07	0.02952	22	XF07	0.03947
27	SY12	0.04284	70	XF13	0.08500
46	39	0.00467	74	73	0.01081
39	20	0.01140	73	63	0.04065
20	SY06	0.03757	63	40	0.01481
20	SY11	0.03757	40	24	0.00846
39	SY14	0.04897	24	WL01	0.04130
52	50	0.00213	24	WL08	0.04130
50	30	0.01081	40	31	0.00472
30	SY03	0.04360	31	WL09	0.04505
30	SY10	0.04360	31	WL14	0.04505
50	3	0.03160	63	57	0.00540
3	SY04	0.02281	57	49	0.00500
3	SY05	0.02281	49	47	0.00037
61	54	0.00468	47	41	0.00401
54	SY09	0.05747	41	17	0.01439
54	SY15	0.05747	17	WL02	0.03540
72	71	0.00218	17	WL07	0.03540
71	69	0.01470	41	WL10	0.04978
69	62	0.01108	47	WL13	0.05380
62	60	0.00247	49	18	0.01800
60	56	0.00355	18	6	0.00874
56	44	0.00610	6	WL04	0.02743
44	38	0.00466	6	WL05	0.02743
38	13	0.01285	18	WL12	0.03616
13	CB01	0.03465	57	42	0.00916
13	CB08	0.03465	42	33	0.00348
38	CB09	0.04750	33	16	0.01113
44	29	0.00862	16	WL03	0.03540
29	5	0.01763	16	WL06	0.03540
5	CB02	0.02591	33	WL11	0.04653
5	CB07	0.02591	42	WL15	0.05001
29	CB12	0.04354	73	37	0.05790
56	CB10	0.05826	37	15	0.01200
60	CB13	0.06181	15	7	0.00705
62	45	0.01093	7	QY01	0.02826
45	35	0.00680	7	QY08	0.02826
35	11	0.01360	15	QY09	0.03531
11	CB04	0.03296	37	26	0.00453
11	CB05	0.03296	26	23	0.00242
35	CB11	0.04656	23	21	0.00272
45	CB15	0.05335	21	19	0.00097
69	48	0.02138	19	8	0.00784
48	32	0.00854	8	QY02	0.02885
32	CB03	0.04545	8	QY07	0.02885
32	CB06	0.04545	19	QY10	0.03669
48	CB14	0.05399	21	14	0.00272
71	70	0.00507	14	4	0.01158
70	68	0.01102	4	1	0.00611
68	65	0.00677	1	QY04	0.01724
65	43	0.01576	1	QY05	0.01724
43	XF01	0.05144	4	QY12	0.02335
43	XF12	0.05144	14	QY14	0.03494
65	59	0.00557	23	12	0.00605
59	34	0.01509	12	9	0.00543
34	XF02	0.04653	9	2	0.00944
34	XF11	0.04653	2	QY03	0.01946
59	53	0.00416	2	QY06	0.01946
53	25	0.01564	9	QY11	0.02889
25	XF03	0.04183	12	QY15	0.03432
25	XF10	0.04183	26	QY13	0.04279

图 1　5 个茸鹿品种(品系)75 个个体的 UPGMA 聚类

Fig. 1　Dendrogram generated by the UPGMA cluster analysis from 75 individuals belonging to five breeds or strains of velvet – deer

更新世以后出现的[9].马鹿是梅花鹿在中东向欧洲和北非扩展过程中而产生的一个新种,这种原始型马鹿到更新世中期又返回中国,在分布扩展过程中,体型逐渐大型化,并形成不同亚种群[9~11].由本研究系统聚类分析的结果可以看出,原始群体来源于天山马鹿的清原品系群与原始群体来源于东北马鹿的乌兰坝品种群聚在一起后,在最后才与代表梅花鹿群体的一支聚在一起.这与李明等[12]利用线粒体 DNA 的细胞色素 b 基因片段序列差异来分析探讨鹿亚种起源与分化的报道相一致,即东北马鹿和天山马鹿分属于马鹿的不同亚种,马鹿和梅花鹿则是鹿亚科(Cervinae)的不同种.

关于我国梅花鹿的亚种分化问题,国内外报道已很多,我国动物学家寿振黄、郑作新等,在梅花鹿的分类问题上已确定中国梅花鹿有 5 个亚种:东北梅花鹿、华北梅花鹿、山西梅花鹿、江南梅花鹿、台湾梅花鹿;但后来又发现了四川梅花鹿亚种.对于我国东北地区的梅花鹿,大多数国内外的研究报道认为只有一个梅花鹿亚种,即东北梅花鹿(或曰乌苏里梅花鹿),但是白庆余教授[13]提出了东北地区的梅花鹿不只是一个亚种,也就是说中国的梅花鹿不是 5 个或 6 个亚种,而是 7 个或 8 个亚种的观点,他认为梅花鹿亚种分化与分类问题需进一步探讨.吉林梅花鹿类型调查组[14]在通过对分布在吉林省境内的梅花鹿进行了全面系统调查研究的基础上认为:吉林梅花鹿至少有 5 个明显不同的类型.本研究依据 RAPD 分析的系统聚类结果,已将 SY、CB、XF 三个梅花鹿群体分开,认为 SY、CB、XF 至少是起源于不同的且存在明显差异的原始群体,这为支持上述这些观点提供了一个佐证.至于东北地区的不同类型的梅花鹿是否可以确立为不同的亚种,以及分布在双阳、长白山、西丰的梅花鹿是否分属不同亚种,尚需进一步研究后得出恰当的结论.

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