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Construction of a California condor BAC library and first-generation chicken–condor comparative physical map as an endangered species conservation genomics resource ☆

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Abstract

To support genomic analysis of the endangered California condor (*Gymnogyps californianus*), a BAC library (CHORI-262) was generated using DNA from the blood of a female. The library consists of 89,665 recombinant BAC clones providing ~14-fold coverage of the presumed ~1.48-Gb genome. Taking advantage of recent progress in chicken genomics, we developed a first-generation comparative chicken–condor physical map using an overgo hybridization approach. The overgos were derived from chicken (164 probes) and New World vulture (8 probes) sequences. Screening a 2.8× subset of the total library resulted in 236 BAC-gene assignments with 2.5 positive BAC clones per successful probe. A preliminary comparative chicken–condor BAC-based map included 93 genes. Comparison of selected condor BAC sequences with orthologous chicken sequences suggested a high degree of conserved synteny between the two avian genomes. This work will aid in identification and characterization of candidate loci for the chondrodystrophy mutation to advance genetic management of this disease.

Keywords: BAC library; California condor; Candidate loci; Comparative physical mapping; Genomics; Overgo hybridization

The application of molecular genetic tools for managing an endangered species plays a conspicuous part in contemporary conservation biology. The California condor (*Gymnogyps californianus*), belonging among the New World vultures (Cathartidae; NWV), is among the first endangered avian species under captive management for which genetic and genomic investigation technologies are being developed and used. Previously, analysis of multilocus DNA fingerprinting data identified three groups consisting of closely related individuals (clans) within the California condor population [1]. A mitochondrial DNA analysis identified four distinct maternal lineages [2]. These findings were utilized to create a hypothetical ancestral generation of California condors that was designated as founders and utilized for population management calculations and recommendations [3]. Additionally, Ralls and colleagues [4] identified a genetic form of chondrodystrophy in condors that appears to be inherited as an autosomal recessive allele. Homozygous affected embryos die in the egg and demonstrate abnormal dwarfism due to a number of bone and cartilage malformations.

Techniques of genome analysis, including physical mapping based on contigs of large-insert (e.g., bacterial artificial chromosome or BAC) clones, linkage studies, and fluorescence *in situ* hybridization, can be applied to the California condor and can proceed more rapidly utilizing results obtained from genome studies and the whole genome sequence of the domestic chicken [5]. Previously, an extensive cytogenetic analysis in the condor identified (i) a chromosome number of

☆ Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. DQ471953 (*CRES0001* microsatellite sequence) and AC171379, AC171743, and AC172166 (CH262-13G5, CH262-21P20, and CH262-48N9 BAC sequences, respectively).

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80 (likely due to an extra pair of microchromosomes relative to chickens) and (ii) information on the centromeres, telomeres, and nucleolar organizer regions [6]. Further, a comparison between condor and chicken macrochromosomes was obtained using individual chicken chromosome-specific paints 1–9 and Z and W (as developed by Griffin and colleagues [7]) on condor metaphase spreads. Except for chromosomes 4 and Z, each of the chicken (GGA) macrochromosomes painted a single condor (GCA) macrochromosome. The GGA4 paint detected homology with two condor chromosomes, viz., GCA4 and GCA9, providing additional proof that the latter are ancestral chromosomes in the birds. The chicken Z chromosome paint hybridized to both Z and W in the condor. This homology suggested that the condor sex chromosomes have not completely differentiated during evolution, unlike the majority of the nonratites studied. Overall, this study provided detailed cytogenetic and basic comparative information on condor chromosomes [6].

To address genome research and genetic management of California condors further, we have begun to develop a genetic map as a prerequisite for identification of candidate loci for the chondrodystrophy mutation. Characterization of candidate loci will enable us to identify carriers of the chondrodystrophy allele and provide tools for improved genetic management of this disease. In this paper, we report the generation of a highly redundant California condor genomic BAC library that was used to construct a first-generation chicken–condor comparative physical map and to identify specific condor BACs carrying candidate genes for chondrodystrophy.

Results

BAC library construction and insert analysis

We constructed the California condor CHORI-262 BAC library using nuclear DNA of a single female. The library was arrayed into 240 384-well microtiter plates, resulting in 92,160 clones, and was subsequently gridded onto five 22×22-cm nylon high-density colony filters for screening by hybridization. Each filter represents about 18,000 distinct BAC clones (equivalent to 48 “384-well” dishes), stamped in duplicate. Estimation of empty wells (i.e., nonrecombinant clones) in the library plates gave an average rate of 2.71% (data not shown). Thus, the library consists of 89,665 recombinant BAC clones, with ~97% containing inserts, and provides ~14-fold coverage of the condor genome. Further description of the condor CHORI-262 library can be seen at http://bacpac.chori.org/library.php?id=222.

BAC library screening with overgo probes

We screened a single filter (~2.8×) of the arrayed library with 172 overgo probes, including 164 from chicken, 2 condor probes, and 6 overgos derived from condor relatives among the NWV (Fig. 1). The probes covered 27 chicken chromosomes including all 12 macrochromosomes (GGA1–10, Z, and W) and 17 of 28 microchromosomes, with an average interval between

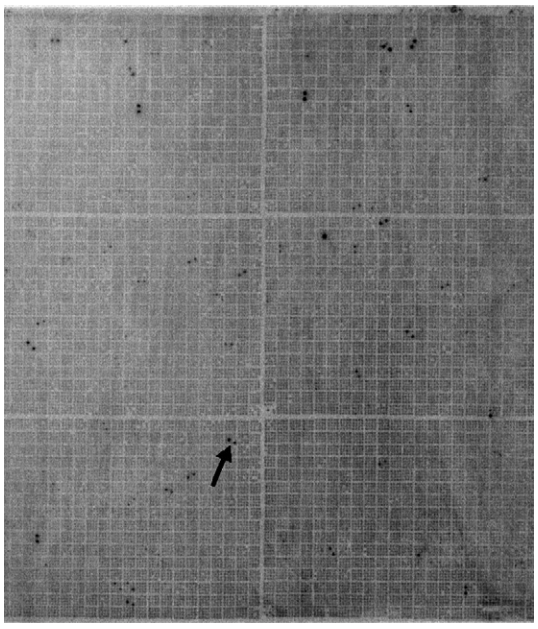

Fig. 1. Example of a California condor BAC library filter hybridized to a pool of 30 overgos. Duplicated spot signals correspond to positive BACs. Among 30 probes, the *MOS* (v-mos Moloney murine sarcoma viral oncogene homolog) probe derived from a condor sequence (GenBank AF339330) was applied and generated one positive clone, CH262-47O3, as shown by an arrow.

probes being roughly 5 Mb in the chicken sequence. The full list of the probes can be found in Supplementary Table 1.

Seventy-seven probes including 76 chicken overgos (46%) and 1 NWV failed to produce positive hybridization. Both California condor probes were successful (though one generated multiple hits), 5 of 6 NWV probes hybridized to California condor BACs, and 88, or 54%, of the chicken overgos were also successful. The probes that were assigned to 1 or more (7 at most) positive BACs are listed in Table 1. The California condor probe *CRES0001* produced 164 positive hits, suggesting that it contains a repetitive sequence, and it was excluded from further analysis. In all, the remaining 93 overgos were assigned to 236 positive BACs, or ~2.5 per probe. A database containing all condor BAC-gene assignments is presented in Supplementary Table 2.

The 6 successful NWV overgos were designed from coding sequences. Among the 88 successful chicken probes, 6 were derived from 5′ and 3′ UTR regions, 2 were derived from 5′ upstream noncoding regions, and 3 overlapped the 3′ end of a codon and 3′ UTR or 3′ downstream noncoding sequences, the remaining 77 probes being designed from coding regions. The 77 probes that were unsuccessful were also designed from both noncoding and coding regions, although noncoding overgos were more frequent (by 19%) in this group. The 93 gene-specific overgos covered 23 chicken chromosomes (12 macro- and 11 microchromosomes), with an average interval of about 10 Mb.

Comparative chicken–condor physical map construction

The cross-species hybridization results have been used to design a preliminary comparative chicken–condor physical

Table 1
Chicken and new world vultures (boldface) overgos hybridized to condor BACs

Locus	Chicken chromosome	Location (Mb)	GenBank Accession No.	Overgo position	No. of positive California condor BACs	Other avian BAC libraries positively tested[a]
CCT2	GGA1	32.4	AW240097	CR, 25…67	6	Chicken
EMP1	GGA1	45.09	BX270611	3′ of CR and 3′ UTR, 382…425	2	Turkey
LGALS4	GGA1	47.87	D00310	CR, 87…129	2	Chicken, turkey
CHUNK1	GGA1	57.6	AJ131560	5′ UTR, 7…44	1	Chicken, turkey
AKR1B1	GGA1	58.8	BG710622	CR, 274…311	1	NT
USP5	GGA1	71.36	BU104255	CR, 1…40	1	Turkey
OCA2	GGA1	123.7	XM_425579	CR, 411…451	2	Turkey
UBE3A	GGA1	124.95	AJ399379	CR, 323…361	3	Chicken, turkey
FUT4	GGA1	177.4	U73678	CR, 1046…1084	1	Chicken, turkey
WNT11	GGA1_random	0.79	D31901	CR, 571…610	1	Chicken, turkey
ACVR2B	GGA2	5.21	U31223	CR, 352…390	4	Chicken, turkey
RARB	GGA2	36.79	X57341	CR, 1712…1751	4	Chicken, turkey
NFX1	GGA2	55.23	BU243588	CR, 63…102	5	Chicken, turkey, zebra finch
EPB41L4B	GGA2	84.33	BU144663	CR, 504…543	1	Chicken, turkey, zebra finch
BAG1	GGA2	85.55	AJ456017	CR, 195…237	2	Chicken, turkey
IRX1	GGA2	87.03	AJ238354	CR, 267…304	1	Turkey
TXNDC4	GGA2	89.18	BU246502	CR, 34…72	2	Chicken
YES1	GGA2	101.1	X12461	CR, 710…747	5	Chicken, turkey
LYN	GGA2 (or UN)[b]	110.38	BU144120	CR, 119…158	4	Turkey
MOS	**GGA2 (or UN)[b]**	**110.41**	**AF339330[c]**	**CR, 145…182**	**1**	**NT**
PENK	GGA2	110.57	BU120351	CR, 124…161	3	Chicken, turkey
VCIP135	GGA2	114.67	Z25845	CR, 20…59	3	Turkey
CA2	GGA2	122.22	X06005	CR, 104…142	1	Chicken, turkey
MYC	**GGA2**	**144.05**	**AY277498[d]**	**CR, 492…529**	**6**	**NT**
PAX5	GGA2	147.4	AJ392389	CR, 437…476	2	Chicken
RYR2	GGA3	33.6	BU362319	CR, 111…150	2	Turkey
FYN	GGA3	64.4	X52841	CR, 19…61	3	Chicken, turkey
BMP5	GGA3	85.69	S83278	CR, 333…372	3	Chicken, turkey
RUNX2	GGA3	107.28	NM_204128	CR, 654…691	7	NT
BTK	GGA4	1.87	AF535118	CR, 1752…1791	5	Chicken, turkey
RRAGA	GGA4	11.23	BU254759	CR, 48…87	2	Chicken
NPY2R	GGA4	21.14	AF309091	CR, 6…43	4	Turkey
NPY5R	GGA4	23.95	AY040844	CR, 1126…1165	5	Turkey
FGF2	GGA4	54.12	M95707	CR, 446…483	4	Turkey
NFKB1	GGA4	61.33	AF000241	CR, 229…266	1	Chicken
FGFR3	GGA4	83.73	M35195	CR, 1585…1626	1	Chicken, turkey
RAG1	**GGA5**	**16.6**	**AY461395[e]**	**CR, 312…354**	**3**	**NT**
ZFP36L1	GGA5	25.24	BM486413	CR, 138…175	1	Turkey
THBS1	GGA5	26.52	U76994	CR, 416…453	5	Chicken, turkey
FOS	GGA5	35.07	M18043	5′ USS, 115…152	3	Turkey
DNCH1	GGA5	46.28	BM487124	CR, 83…122	1	Chicken, turkey
BRF1	GGA5	49.56	BU255571	CR, 121…158	3	Turkey
BMP4	GGA5	55.23	X75915	CR, 528…567	5	Chicken, turkey
SUPV3L1	GGA6	10.13	CD219024	CR, 315…354	2	Turkey
CYP17A1	GGA6	22.45	M21406	CR, 1223…1240	2	Chicken, turkey
CD28	GGA7	14.25	X67915	CR, 149…188	2	Chicken, turkey
NRD1	GGA8	24.42	AL584107	CR, 44…83	2	Chicken
PAX3	GGA9	5.76	AB080581	CR, 20…59	1	NT
PER2	**GGA9**	**8.14**	**AY277549[f]**	**CR, 1300…1337**	**1**	**NT**
SEC11L1	GGA10	1.65	AW355399	CR, 17…54	5	Turkey
CHRNB4	GGA10	4.4	J05643	CR, 110…149	3	Chicken, turkey, zebra finch
TJP1	GGA10	6.82	BU401762	CR, 14…53	2	Chicken, turkey
TRPM7	GGA10	11.41	AJ397352	5′ UTR, 2…43	3	Chicken, turkey
AGC1	GGA10	13.63	U78555	5′ UTR, 125…164	2	Chicken, turkey
RPL4	GGA10	19.19	BI392283	CR, 47…84	5	Chicken, turkey
MC1R	GGA11	19.01	D78272	CR, 645…683	3	Chicken, turkey
RAF1	GGA12	5.02	X07017	CR, 74…113	4	Turkey
NINJ1	GGA12	6.67	BU132812	CR, 393…432	1	Chicken
GABRA1	GGA13	2.36	X54244	CR, 1503…1542	3	Chicken, turkey
OVM	GGA13	9.54	J00894	5′ USS, 310…349	1	Turkey
ACSL6	GGA13	15.67	BH126249	CR, 79…117	4	Chicken, turkey

(continued on next page)

Table 1 (*continued*)

Locus	Chicken chromosome	Location (Mb)	GenBank Accession No.	Overgo position	No. of positive California condor BACs	Other avian BAC libraries positively tested [a]
NR3C1	GGA13	16.41	AY029202	CR, 3…40	2	Chicken, turkey
ALDH2	GGA15	0	BI066306	CR, 207…244	4	Chicken
FBXW2	GGA17	0.067	AJ452913	CR, 408…446	2	Chicken
ZNF297B	GGA17	0.2	BU237708	CR, 658…700	2	Chicken
HSPA5	GGA17	0.85	M27260	CR, 118…155	6	Chicken
CEP1	GGA17	2.05	BU474772	CR, 278…315	1	NT
BRD3	GGA17	3.24	AJ395054	CR, 353…390	3	Chicken
USP20	GGA17	5.15	BU460884	CR, 34…71	2	Chicken
ENG	GGA17	5.84	BU414929	CR, 891…929	1	Chicken, turkey
DBC1	GGA17	6.8	BI391514	CR, 68…105	5	Chicken, turkey
AANAT	**GGA18**	**4.2**	**AY339825** [f]	**Exon 1 and start of intron 1–2, 89…126**	**2**	**NT**
FASN	GGA18	4.73	J03860	5′ UTR, 9…46	2	Chicken, turkey
MYBBP1A	GGA19	2.69	AI980741	CR, 251…293	2	NT
AGRN	GGA21	2.63	M94271	5′ UTR, 7…44	2	Chicken, turkey
PRNP	**GGA22**	***0.43***	**AF157954** [g]	**CR, 348…385**	**2**	**NT**
PVRL1	GGA24	3.96	X85516	3′ end of CR and start of 3′ DSS, 7…46	2	Chicken, turkey
PAFAH1B2	GGA24	4.89	AJ394959	CR, 230…269	1	Chicken, turkey
TAGLN	GGA24_random	0.14	M83105	CR, 97…134	1	Chicken, turkey
ACCN1	GGA27	0.28	BU140225	CR, 699…736	1	Turkey
PGM5	GGAZ	10.75	BM485657	3′ UTR, 329…366	2	Chicken, turkey, zebra finch
JMJD2C	GGAZ	12.24	BU248770	CR, 153…193	1	Chicken, turkey, zebra finch
ZA20D2	GGAZ	16.53	AJ448872	CR, 290…327	4	Chicken, turkey, zebra finch
PCSK5	GGAZ	17.7	BU265867	CR, 73…110	1	Chicken, turkey, zebra finch
AUH	GGAZ	19.46	BU132994	CR, 226…265	3	Chicken, turkey, zebra finch
TAL2	GGAZ	26.85	BU214478	CR, 205…242	1	Chicken, turkey, zebra finch
AKAP2	GGAZ	31.89	BU223228	CR, 112…151	1	Chicken, turkey, zebra finch
NFIB	GGAZ_random	1.78	BU250474	CR, 10…47	1	Chicken, turkey, zebra finch
CCDC2	GGAZ_random	2.65	BU211538	CR, 178…217	2	Chicken, turkey, zebra finch
IKBKAP	GGAZ_random	4.27	AJ453239	CR, 218…257	2	Chicken
WDR40A	GGAZ_random	7.08	BM488521	CR, 35…74	3	Chicken
CNTFR	GGAZ_random	7.7	BU139458	CR, 74…111	1	Chicken, turkey, zebra finch
UBQLN1	GGAZ_random	12.62	BI066615	CR, 179…216	2	Chicken, turkey, zebra finch
UBE2R2	GGAW_random	0.18	BU122359	CR and 3′ UTR, 715…752	1	Chicken, turkey, zebra finch

CR, coding region; UTR, untranslated region; USS, upstream sequence; DSS, downstream sequence; NT, not tested.

[a] According to information from http://poultry.mph.msu.edu/resources/Resources.htm and Ref. [11].

[b] The overgo also aligns with an unknown (UN) chromosome sequence that is yet to be assigned to a named chromosome.

[c] California condor (*Gymnogyps californianus*).

[d] Black vulture (*Coragyps atratus*).

[e] Turkey vulture (*Cathartes aura*).

[f] Greater yellow-headed vulture (*Cathartes melambrotus*).

[g] Andean condor (*Vultur gryphus*).

map using the MapChart format (Fig. 2). The map shows locations of 93 genes (88 chicken and 5 NWV) and is also available in the GenomePixelizer format (Supplementary Fig. 1).

To confirm the efficiency of our interspecies hybridization strategy and obtain nucleotide sequences for condor genes, selected positive BACs were sequenced (GenBank AC171379, AC171743, and AC172166). The clones harbored sequences of the genes *AGC1* (CH262-21P20, AC171743) and *RUNX2* (CH262-13G5, AC171379; CH262-48N9, AC172166) and showed homology to the appropriate chicken chromosomes (GGA10 and GGA3, respectively). A preliminary comparative sequence analysis of a 32-kb sequence containing the condor *AGC1* gene, a potential candidate gene for chondrodystrophy, using BLAST (http://www.ncbi.nlm.nih.gov/blast/) against genome databases of the chicken, human, mouse, and zebrafish (Table 2) showed highest homology with the chicken sequence, as expected.

Discussion

Construction of a genomic BAC library for a given species is considered a milestone in the development of specific genomic resources that can be applied to a wide spectrum of research opportunities, especially for an organism poorly studied, to date, from the standpoint of molecular genetics and genomics. The 14-fold coverage provided by this condor genome BAC library implies a very high probability that the library includes almost any condor sequence. Use of DNA

Table 2
Comparison of the condor *AGC1* gene sequence inferred from CH262-21P20 (AC171743) with orthologs in other species

Species	Gene length (kb)	Score (bits)	*E* value
Condor	32	22470	0.0
Chicken	43	1625	0.0
Human	71	254	2×10^{-63}
Mouse	62	212	1×10^{-50}
Zebrafish	20	114	2×10^{-21}

Score and *E* value for condor derived from BLAST against AC171743. Comparison with orthologs in other species was done using BLAST against genome databases.

from a condor female for library development provided representation of large-insert clones for all autosomes and both sex chromosomes, Z and W.

One of the direct applications of such a BAC library is the creation of a comparative physical map aligned to a whole genome sequence available from another related species. We used the chicken draft sequence for cross-species library screening and comparative BAC-based mapping of the California condor genome. Comparison of the chicken and condor genomes is facilitated by the facts that both species belong to the same class of Aves and have similar chromosome organizations, with 2*n* being 78 in the chicken and 80 (possibly one extra microchromosome pair) in the condor [6]. The two avian species also have similar-sized genomes. The condor genome size estimate (haploid *C* value using Feulgen densitometry) is 1.51 pg [8,9] (http://www.genomesize.com/result_species.php?id=893), equivalent [10] to 1.48 Gb, while the chicken *C* value is 1.25 pg [9] (http://www.genomesize.com/result_species.php?id=906), which corresponds to 1.22 Gb.

We confirmed the quality and representation of our library by filter hybridization using highly specific overgo probes. Although only one of five BAC filters was employed, 93 probes that hybridized generated 236 BAC-gene assignments, or 2.5 per probe. This was close to the number expected from the 2.8-fold redundancy of one filter. Furthermore, comparison of three selected condor BAC sequences with the whole chicken genome sequence additionally suggested a high degree of conserved sequence homology between these two avian genomes.

One of two California condor probes, *CRES0001*, was excluded from the comparative map since its sequence did not match any chicken sequence, and it generated multiple hits to the condor BAC library. *CRES0001* was generated by sequencing a clone from an enriched California condor microsatellite library (unpublished data). Examination of the *CRES0001* (DQ471953) sequence resulted in identification of the simple pentanucleotide repeat $[(TTGTG)_n]$ at positions 271...328, while the overgo (5′-GTGTAGGTCTCTAGCCAA-TGTCTCAGGTACCCAGGGAT-3′) was derived from the *CRES0001* sequence at positions 78...115. Thus, the probe may represent an unknown longer repetitive element that is not related to the adjacent microsatellite. Multiple hits derived from a single overgo probe were previously observed in screening chicken BACs with four probes specific for microsatellite loci *MCW0038* (L43677), *MCW0081* (L43636), *MCW0214* (G31991), and *LEI0126* (X82799) (M.N. Romanov and J.B. Dodgson, unpublished data). These repetitive chicken probes were also designed using microsatellite-flanking sequences. While the primer pairs used to genotype these microsatellites presumably are unique, it appears that larger flanking sequences used for overgo design detect repetitive elements in these cases.

The chicken probes for *CHUNK1* and *OCA2,* which are located on GGA1 a great distance apart, hybridized to the same condor BAC, CH262-5D3. This may be evidence of an intrachromosomal rearrangement (transposition or inversion) in the appropriate condor chromosome relative to chicken, although additional mapping will be required to confirm it. Two other probes, for *PAFAH1B2* and *TAGLN,* also hybridized to the same condor clone, CH262-43E17. Although in the current chicken genome assembly (http://genome.ucsc.edu/cgi-bin/hgGateway?clade=vertebrate&org=Chicken&db=0&hgsid=41250285) these two genes are respectively assigned to GGA24 and a random, nonaligned piece of the same chromosome (GGA24–random), they are expected to be close to each other in chicken. This is supported by the fact that both probes also hybridized to identical clones in the chicken BAC library (http://poultry.mph.msu.edu/resources/Resources.htm#bacdata).

Chicken overgo probes hybridized to condor BACs with a 54% success rate, despite ~100 million years of evolutionary divergence between the two avian lineages and their last common ancestor [11]. Given that we screened only ~2.8× coverage of the genome, it is expected that 6% of the probes would fail due to the random chance that the filter lacked any appropriate BAC. The remaining probes presumably failed mostly due to lack of homology between condor and chicken sequences. Many of the probes that were successful were previously observed to work in screening turkey and zebra finch BAC libraries [12] (Table 1). Our data on the efficiency rate for the chicken–condor interspecies hybridization are consistent with those obtained in the chicken–zebra finch (48%) and zebra finch–turkey (68%) comparisons [12]. The passerine lineage (zebra finch) and the ciconiiforms (condor) are both among the Neoaves and thus should have a common divergence time from land fowl (chicken and turkey) [12]. The overgos used to date for condor were not specifically designed for the purpose of multispecies hybridization and sequence comparison. Further design improvements can increase the success rate, if overgo design criteria will more rigorously follow recommendations for so-called “universal” probes derived from highly conserved regions [13,14]. Kellner and colleagues [14] demonstrated a very high success rate of universal probes in turkey (98%), zebra finch (85%), and emu (80%). These probes can be a valuable resource for isolating orthologous genomic regions simultaneously from multiple BAC libraries.

A heritable embryonic lethal chondrodystrophy phenotype has been identified that segregates in the affected pedigree as a Mendelian character in a manner consistent with an autosomal recessive mode of inheritance [4]. The genes

anchored to the California condor BAC library include several involved in bone and cartilage formation that could be candidates for the condor chondrodistrophy: *AGC1* (aggrecan 1, chondroitin sulfate proteoglycan 1, large aggregating proteoglycan; causes chondrodistrophy in the chicken (nanomelia) [15] and turkey [16]), GGA10; *CHUNK1* (chondrocyte unknown protein), GGA1; *CA2* (carbonic anhydrase II), GGA2; *BMP5* (bone morphogenetic protein 5), GGA3; *RUNX2* (runt-related transcription factor 2), GGA3; and *BMP4* (bone morphogenetic protein 4), GGA5. The

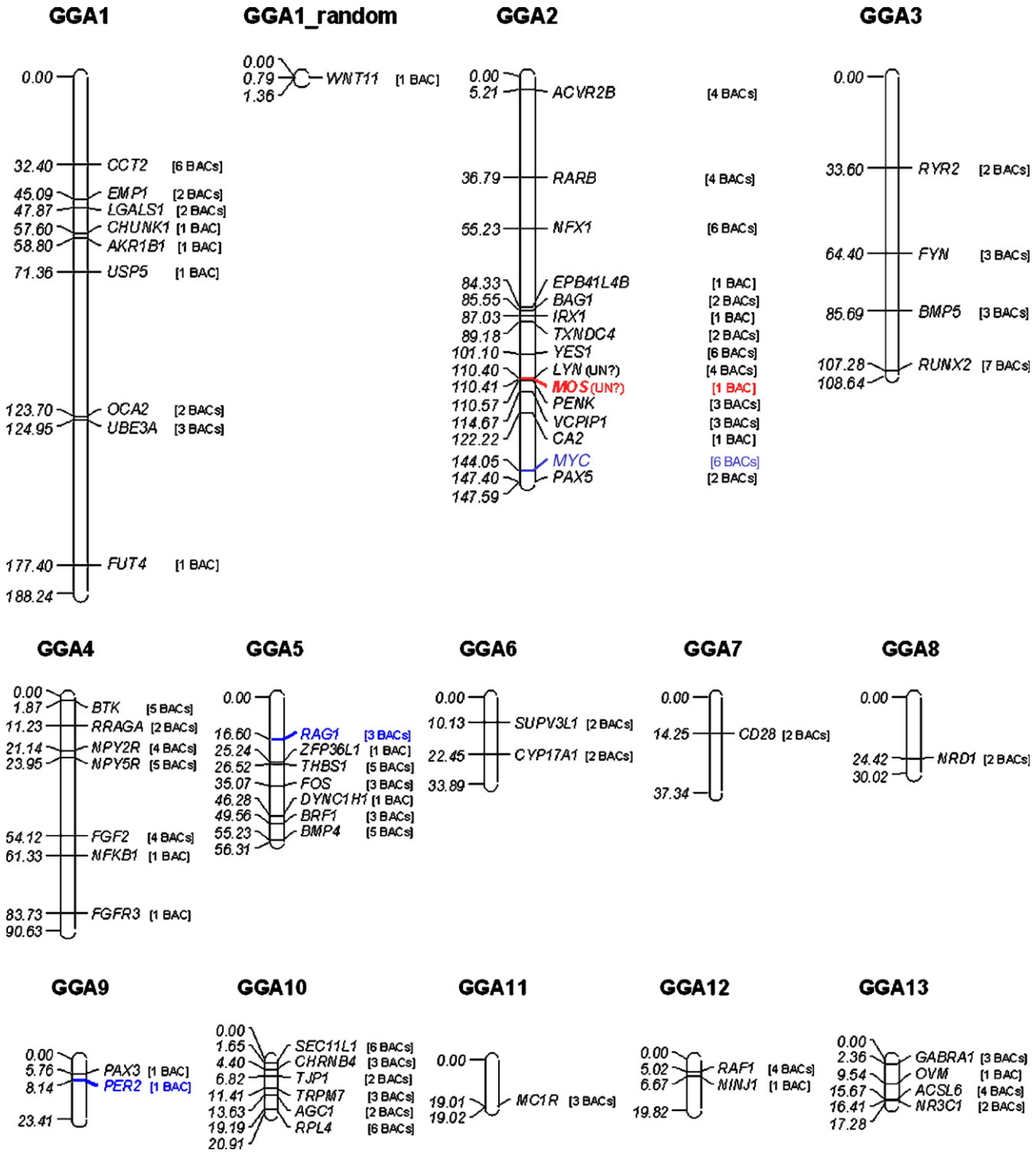

Fig. 2. Chicken chromosome map (in Mb) created with MapChart [19] and showing locations of the 93 genes aligned with the California condor BAC clones (numbers in brackets). Colored symbols indicate chicken genes that were anchored to condor BACs using California condor (red) and New World vulture (blue) sequence-derived overgos. Probes for two genes on GGA2, *LYN* and *MOS,* also align with an unknown (UN) chromosome sequence generated by the International Chicken Genome Sequencing Consortium [5] that is yet to be assigned to a named chromosome.

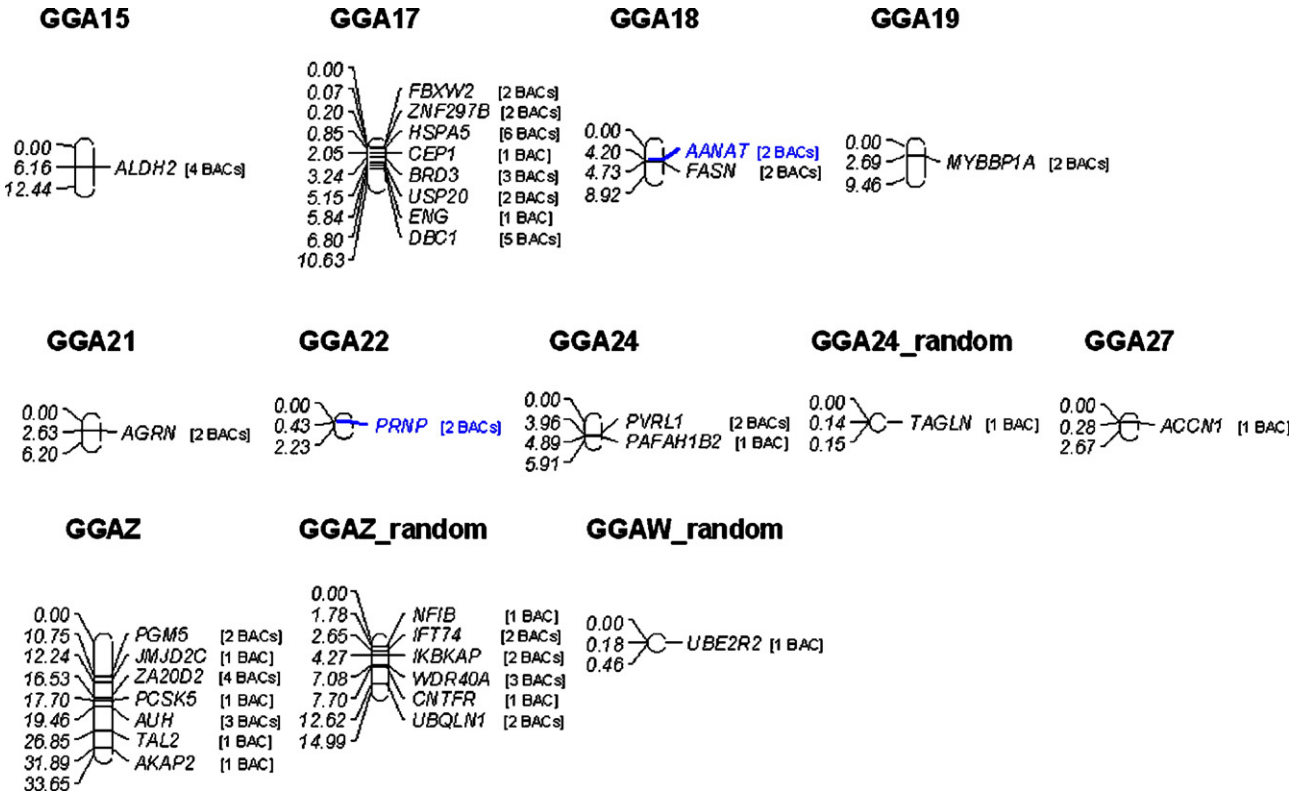

Fig. 2 (*continued*).

condor BAC clones containing these candidate genes will be used as a primary source of sequence information in further candidate gene association studies.

In the long-term perspective, construction of genetic and physical maps of the California condor and determination and characterization of candidate loci for the chondrodystrophy mutation will be critical steps in the effort to develop genotype assays for the chondrodystrophy allele and provide such information for population management. A diagnostic method to distinguish heterozygous mutant carriers from noncarriers will enable managing the potential impacts of chondrodystrophy on both captive and wild subcomponents of the condor population. Due to the extreme bottleneck in the condor population [4], it is likely that other deleterious alleles will be present among existing families and require genome analysis to improve management decisions. In this sense, a physical map also prepares the way to address heritable defects that might be identified in the future. Furthermore, loci conferring sensitivity or resistance to infectious disease (e.g., West Nile virus) may be scrutinized with a condor map of sufficient resolution. Efforts undertaken to understand better the genetic makeup of California condors will serve as a model for other species.

Despite the evolutionary divergence between avian taxa, their genomes can be analyzed and compared by probing with a set of overgos, as shown in this study and other reports (e.g., [12,14]). The only resource required would be high-quality, gridded BAC libraries for avian genomes of interest. This argues in favor of continued construction of BAC libraries in birds. Additional information on the California condor genome project can be found at http://cres.sandiegozoo.org/projects/gr–condor–genome.html.

Materials and methods

BAC library construction

The CHORI-262 BAC library of the California condor was generated at the BACPAC Resources Center, CHORI (http://bacpac.chori.org/). The preparation of the library followed the general cloning approach developed by Osoegawa and colleagues [17]. DNA was isolated from 1 ml of condor blood obtained at the San Diego Zoo as part of routine health monitoring. The sample was derived from a 14-year-old female condor "Molloko" (Studbook No. 45, ISIS 888071) that was not a carrier of the chondrodystrophy mutation. Blood was mixed with low-melting agarose to embed all-nucleated blood cells. Agarose-embedded cells were left to solidify in a plug mold at the concentration 5×10^6 cells per plug (approximately 10 μg). High-molecular-weight (HMW) DNA was extracted using SLS proteinase K lysis buffer as previously described [17]. The HMW DNA was partially digested with a combination of *Eco*RI restriction enzyme and *Eco*RI methylase and size fractionated by pulsed-field electrophoresis. DNA fragments from the 170- to 250-kb size fraction were cloned into the pTARBAC2.1 vector between the two *Eco*RI sites. The ligation products were transformed into DH10B (T1 resistant) electrocompetent cells (Invitrogen).

BAC library screening

Screening of the arrayed library was carried out using the CHORI protocol (http://bacpac.chori.org/overgohyb.htm), overgo probes, and a four-dimensional filter hybridization approach [18]. In brief, overgos (overlapping oligo probes) were derived from specific sequence regions in known genes or markers. They were synthesized by annealing two oligonucleotides that have an 8-bp overlap, followed by labeling *in vitro* with [α-^{32}P]dATP and dCTP nucleotides. Use of

overgos facilitated pooling strategies because the melting temperatures for all probes were chosen to be nearly the same.

A collection of overgos derived from the publicly available chicken and New World vulture nucleotide sequences (http://www.ncbi.nlm.nih.gov/) was arranged for three-dimensional screening by plates, rows, and columns. We used a set of 172 probes and pooled 28–36 overgos including one common anchor probe for a single hybridization. Sequences of the overgo probes are available from the authors upon request. Most of them were previously used to screen chicken, turkey, and zebra finch BAC libraries [12,18] (Table 1). For probe design, selected sequence alignments were performed and deposited in the EMBLALIGN database (http://srs.ebi.ac.uk/srs6bin/cgi-bin/wgetz?-page+query+-libList+EMBLALIGN+-newId) under Accession Nos. ALIGN_000578, ALIGN_000597, and ALIGN_000636. Each probe was assigned to a number of positive BAC clones common for a particular intersection of plate, row, and column. In total, we had 17 hybridizations including 5 plate, 6 row, and 6 column hybridizations. To improve accuracy of library screening, we complemented it with 6 additional, diagonal hybridizations. As a further improvement of the Romanov and colleagues [18] screening protocol, we labeled all 172 probes at once and subsequently did four hybridization rounds with 5 or 6 hybridizations at a time. This allowed us to spend a significantly smaller amount of [α-^{32}P]dATP and dCTP nucleotides (1/24 if compared to the standard labeling protocol [18]) and to perform a total of 23 hybridizations in 2 weeks.

For the screening trial, we chose one of five high-density library filters, the one containing clones CH262-1A1 to CH262-48P24. After the 23 filter hybridizations by overgo probes, positive hits in the condor BAC library were scored using ArrayVision software (Imaging Research, Inc., now part of GE Healthcare: http://www.imagingresearch.com), deconvoluted using an in-house Microsoft Access-based program [18], and categorized as probable, tentative, or weak positives as described elsewhere [18]. Selected condor microsatellite clone and positive BAC clone nucleotide sequences were obtained at Genetic Identification Services (http://www.genetic-id-services.com/) and the NIH Intramural Sequencing Center (http://www.nisc.nih.gov/) and deposited with GenBank (DQ471953, AC171379, AC171743, and AC172166). A map of BAC-gene assignments to individual chicken chromosomes was constructed in two formats using MapChart [19] and GenomePixelizer [20] software.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2006.06.005.

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