the muscular system and provide metric coordinates for sensation. As the initially slow-conducting thalamo-cortical and long-range motor-sensory connections develop[3,25], the temporal prolongation of sensory feedback by the self-generated spindle may assist in strengthening the new connections, thus contributing to the establishment of sensorimotor coordination. □

Methods

Neocortical recordings were made from freely moving and anaesthetized neonatal rats (post-natal days (P) 1–8; $n = 45$)[27]. In freely moving pups ($n = 7$), 4–8 tungsten wires (20 μm in diameter) with 100–300 μm vertical tip separation were implanted at age P4 (−0.5–1.5 mm posterior to the bregma and 1.5–2.0 mm from the midline) under ice-cooling anaesthesia. In 17 additional pups (P1–P6), two anchor bars were fixed to the skull under isoflurane anaesthesia. After recovery, the head was restrained by the skull bars and the body was surrounded by a cotton nest, mimicking the presence of littermates[28,29]. An array of eight electrodes was placed in the same cortical depth in S1 so that at least some of them recorded units. A water heater placed under the nest kept the body temperature constant at 35 °C. Acute recordings ($n = 21$) were performed under urethane anaesthesia (1–1.5 g kg⁻¹). For simultaneous recording of field potential and multi-unit activity, silicon probes[30] or tungsten wires (20 μm in diameter) were used. The silicon probe (16 recording sites with 100 μm separation) was inserted vertically into the brain so that simultaneous recordings could be made from the somato-sensory cortex at various depths. Whole-cell patch-clamp recordings were performed in the vicinity of the extracellular electrode ($n = 9$ rats)[27]. Morphological identification of the recorded cells was performed by adding biocytine (0.4%) to the pipette solution. Pyramidal cells with typical apical dendrite ramifying close to the brain surface were found in layer V ($n = 4$). Data were acquired at a sampling rate of 20 kHz and analysed off-line. Oscillatory events were detected by filtering the raw data in respective frequency ranges, identifying periods of high power of sufficient duration and then detecting the troughs. Multi-unit bursts were detected from the discriminated spike trains. Movements were recorded at 30 frames per second and reviewed frame-by-frame. The beginning and the end of all motor movements, including phasic and tonic, local and generalized movements were detected by eye. For finer temporal resolution, movements of the forelimb, hindlimb and body were detected by miniature earphones placed on the limbs. Sensitivity was sufficient to detect the smallest visible movements, including respiration-related displacement of the limbs (Fig. 1). Further details are provided in the Supplementary Methods.

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A physical map of the chicken genome

John W. Wallis[1], Jan Aerts[2], Martien A. M. Groenen[2], Richard P. M. A. Crooijmans[2], Dan Layman[1], Tina A. Graves[1], Debra E. Scheer[1], Colin Kremitzki[1], Mary J. Fedele[1], Nancy K. Mudd[1], Marco Cardenas[1], Jamey Higginbotham[1], Jason Carter[1], Rebecca McGrane[1], Tony Gaige[1], Kelly Mead[1], Jason Walker[1], Derek Albracht[1], Jonathan Davito[1], Shiaw-Pyng Yang[1], Shin Leong[1], Asif Chinwalla[1], Mandeep Sekhon[1], Kristine Wylie[1], Jerry Dodgson[3], Michael N. Romanov[3], Hans Cheng[4], Pieter J. de Jong[5], Kazutoyo Osoegawa[5], Mikhail Nefedov[5], Hongbin Zhang[6], John D. McPherson[7], Martin Krzywinski[8], Jacquie Schein[8], LaDeana Hillier[1], Elaine R. Mardis[1], Richard K. Wilson[1] & Wesley C. Warren[1]

[1]*Genome Sequencing Center, Washington University School of Medicine, Campus Box 8501, 4444 Forest Park Avenue, St Louis, Missouri 63108, USA*
[2]*Animal Breeding and Genetics Group, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands*
[3]*Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan 48824, USA*
[4]*Avian Disease and Oncology Laboratory, Agricultural Research Service, USDA, East Lansing, Michigan 48823, USA*
[5]*Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr Way, Oakland, California 94609, USA*
[6]*Department of Soil and Crop Sciences and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, Texas 77843, USA*
[7]*Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza Houston, Texas 77030, USA*
[8]*Genome Sciences Centre, British Columbia Cancer Agency, 570 West 7th Avenue, Vancouver, British Colombia, V5Z 4E6, Canada*

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Strategies for assembling large, complex genomes have evolved to include a combination of whole-genome shotgun sequencing and hierarchal map-assisted sequencing[1,2]. Whole-genome maps of all types can aid genome assemblies, generally starting with low-resolution cytogenetic maps and ending with the highest

resolution of sequence. Fingerprint clone maps are based upon complete restriction enzyme digests of clones representative of the target genome, and ultimately comprise a near-contiguous path of clones across the genome. Such clone-based maps are used to validate sequence assembly order, supply long-range linking information for assembled sequences, anchor sequences to the genetic map and provide templates for closing gaps. Fingerprint maps are also a critical resource for subsequent functional genomic studies, because they provide a redundant and ordered sampling of the genome with clones[3]. In an accompanying paper[4] we describe the draft genome sequence of the chicken, *Gallus gallus*, the first species sequenced that is both a model organism and a global food source. Here we present a clone-based physical map of the chicken genome at 20-fold coverage, containing 260 contigs of overlapping clones. This map represents approximately 91% of the chicken genome and enables identification of chicken clones aligned to positions in other sequenced genomes.

For large genome sequence assemblies so far, contiguous clone-based maps provide the framework for organizing the sequence[5,6]. With the increasing emphasis on whole-genome shotgun assemblies, integration of mapping data throughout the assembly and finishing process is key to improving efficiency and assuring accuracy equivalent to human genome standards. The basis of map construction for any genome is the reliance on random breakage of genome structure and the subsequent ordering of these genome pieces. Once assembled, physical maps are a key resource in the dissemination of clones by known location for use in all disciplines of biology.

A large number of chicken genomic resources are available, including a collection of large-insert bacterial artificial chromosome (BAC) clone libraries[7,8]. These libraries were made from the inbred red jungle fowl 256 (RJF, *G. gallus*) strain used in whole-genome sequencing and from the white leghorn, a domestic breed selected for egg production. Meiotic linkage maps for three mapping populations were used to develop a consensus map containing about 2,000 sequence-tagged site (STS) markers[9,10]. Markers that were assigned to BACs were a key resource in map construction.

We constructed a physical clone map from 154,560 fingerprints of *G. gallus* BACs. Automated band recognition software identified restriction digest fragments[11]. A small fraction (15%) of these fingerprints were lost due to empty lanes, empty insert clones, incomplete restriction enzyme digestion, or failure by the software to recognize lanes. The FPC program processed the remaining 130,486 fingerprints[12]. Automated overlap evaluation of these fingerprints generated 6,509 contigs using a Sulston score[13] threshold of 1×10^{-17}, tolerance of 7. The Sulston score approximates the probability of two clones realizing a given number of fingerprint band matches by coincidence. These parameters left 51,954 singleton clones not assigned to a contig. The final automated step refined clone order using the program CORAL[14]. CORAL has an improved clone-ordering algorithm proven effective when applied to individual FPC contigs.

Subsequent to automated map assembly, manual review is an essential step in building a reliable fingerprint map. Although a required element in high-throughput generation of fingerprints, automated identification of bands produces band-calling errors that cannot be completely eliminated. These errors propagate during clone ordering and result in incorrectly assembled map contigs. Dispersed and tandem sequence repeats can also confound software programs, collapsing different regions into a complex mix of falsely overlapping clones. Finally, there is currently no automated procedure for merging related contigs. Therefore, we visually examined the fingerprint images of digested clones in each contig to limit errors in automated contig construction. Clone order errors were resolved and fingerprints not meeting expectations for band pattern

were removed from contigs and returned to the singleton pool. To merge overlapping contigs, we relaxed the Sulston score threshold to 1×10^{-10} and then to 1×10^{-7}. Clones comprising the terminal ends of contigs were allowed to join other contigs after manual review. We added individual singleton clones to contigs as needed to increase coverage of sparse regions. If a clone did not provide further band information, it was left in the singleton pool. Of the initial 6,509 contigs, 95% were joined to produce 320 contigs.

Markers are essential for anchoring the fingerprint map to chromosomes, and they also permit validation of contig integrity. A limited number ($n = 911$) of STS markers have been mapped to fingerprinted RJF clones; 730 of these have chromosome placements[15]. The white leghorn fingerprint database, however, is a rich source of marker data, with 1,830 markers (1,717 assigned to chromosomes). To increase the number of links to the genetic map, we added the fingerprints of 49,805 white leghorn BAC clones to the physical map. We assigned 29,663 white leghorn clones to contigs, based on the restrictive condition that each of these clones had a fingerprint match to at least five RJF contig clones. Manual inspection confirmed the positions of 2,244 white leghorn clones having assigned markers. The white leghorn clones were used for their marker information only; their fingerprints were not used to join RJF contigs.

We also took advantage of the draft sequence assembly to refine further the fingerprint map. The assembly and fingerprint map are linked by 128,523 BAC-end sequences (BES). After requiring a minimum of six end-sequence links, a total of 189 fingerprint contigs were reliably assigned to the sequence assembly. Some of the contigs left unassigned were linked to assembly contigs in a topologically impossible manner, indicating errors in the assembly or fingerprint map. For example, a pair of contigs cannot be correctly linked by their ends if the middle portions are linked to different contigs. Potentially incorrect regions of the assembly and fingerprint maps were reciprocally examined, revealing 14 misassembled fingerprint contigs that were subsequently split apart. These cases were primarily due to contaminated clones missed during the manual review process.

The assembly also provided preliminary order to 3,557 singleton clones not assigned to fingerprint contigs. Small groups of overlapping singletons often served as bridges that could join contigs. Merges suggested by the assembly were examined and made only when supported by fingerprint data, although a Sulston score of 1×10^{-6} was accepted with the additional sequence evidence. A summary of clone distribution in the final 260 contigs is shown in Table 1.

The genetic map was then used to anchor the fingerprint contigs to chromosomes. We determined the distribution of contigs on each chicken chromosome using a simple plurality of chromosome assignments for the marker–clone pairs in each contig. Markers assigned to white leghorn clones were not considered unless the contig location of the clone had been manually confirmed. A total of

Table 1 Summary of clone distribution in the physical map

Number of clones	180,291
Number of singletons	47,070
Number of contigs	260
Contig size distribution	
> 200 clones	105
101–200 clones	32
51–100 clones	33
26–50 clones	32
10–25 clones	35
3–9 clones	22
2 clones	1

Singletons include 19,617 white leghorn and 27,453 RJF clones.

186 contigs were assigned in this way. Linkage of the fingerprint map to the sequence assembly provides positional information for many contigs. All 125 contigs suggested by the assembly to be collinear had consistent chromosome assignments. An additional seven contigs lacking independent marker data were given chromosome assignments based on their linkage to an assigned FPC contig. Finally, markers positioned on assembly contigs by BLAST sequence comparison provided additional support of the chromosome assignments and allowed localization of an additional 33 fingerprint contigs based on their linkage to assembled sequence. A summary of the 226 contigs mapped to chromosomes is found in Table 2.

A minimally overlapping set of clones spanning the map contigs is useful for comparative genomic studies[16], as a source of specific cloned sequence, and as a reliable estimator of the physical length of each contig. We used the software Minilda (http://mkweb.bcgsc.ca/minilda/) to select clones with the goals of maximizing the amount of unique content in each clone selection, limiting excessive overlap, avoiding gaps between adjacent selections, and avoiding clones with fingerprint bands unconfirmed by overlapping clones. Our estimated minimum tiling path set consists of 9,210 BAC clones with an average clone overlap of 77 kilobases (kb) (http://mkweb.bcgsc.ca/chicken/images/?list = 003). Estimates of the physical size of each contig were made and the totals per chromosome are listed in Table 2. The total amount of sequence represented in fingerprint contigs is 0.97 gigabases (Gb), or 91% of the current sequence assembly[4].

Low coverage BAC-based fingerprint maps of the chicken genome were recently published[7,8]. The maps represent preliminary efforts to generate resources for the community, such as region-specific BAC clones as templates for polymorphic marker development. Ren et al.[7] report 2,331 contigs, estimated to cover ~7.5 ×

genome equivalents, with 367 markers used to anchor only 11% of the contigs. The estimated 3.6 × genome coverage by Aerts et al.[8] was insufficient for accurate comparisons of clone distribution by contig. Both of these reports represented progress towards developing clone resources for functional genomic studies and for improving knowledge of local clone order for selected chromosomes; for example, G. gallus chromosome 10 (GGA10)[8]. However, neither report created the comprehensive coverage required for sequence assembly validation or provided a sufficient resource to pick tiling paths of reduced genome representation. Our manually curated map of 180,291 clones and 260 contigs represents a major advance upon these early efforts, while at the same time incorporating the identical libraries. In particular, this map integrates an additional, much larger BAC library (CHORI-261), 2,628 STS markers, 128,523 BAC-end sequences, the chicken whole-genome sequence assembly, and much higher clone redundancy. These additional resources allowed us to improve greatly clone contig distribution as demonstrated by a 100-fold increase in large contigs, defined as >200 clones per contig (see Table 1). The physical map is available in standard formats, such as CMAP (http://gmod.wustl.edu/cgi-bin/cmap/viewer) and GBROWSE (http://www.animalsciences.nl/ChickFPC). It can also be downloaded directly from http://genome.wustl.edu/pub/groups/mapping/fpc_files/chicken/.

Combining physical maps with a whole-genome, shotgun-based strategy provides an ideal blueprint for sequence assembly[17]. Critically, the physical map with corresponding marker information allowed reciprocal error checking of the physical map and the whole-genome shotgun assembly while at the same time providing invaluable long-range linking information. This allowed anchoring, ordering and orienting the sequence along the chicken genome. The clone-based map now also provides a key resource for moving towards improving the chicken genome sequence, filling gaps and sorting out difficult regions of the chicken genome. Future avian molecular genetic research will be greatly aided by this BAC-based map and accompanying minimum tiling path. □

Methods

Fingerprinting

Isolation of plasmid DNA from individual BAC clones was performed according to the manufacturer's recommendations (Brinkman Instruments). The HinDIII digestion and agarose gel fractionation of BAC clones from TAM31, TAM32 and TAM33 (ref. 7) (http://hbz.tamu.edu/bacindex.html), CHORI-261 (http://bacpac.chori.org) and WAG[8] (http://www.zod.wau.nl/abg/) libraries were as described previously[18]. After fluorescent staining and imaging, gel images were analysed using IMAGE software[19] (http://www.sanger.ac.uk/Software/Image), followed by automated band calling with BandLeader[11].

BAC end sequencing

End sequences of BAC plasmids were obtained using previously established methods with minor modifications[20]. Resulting traces were transferred into a database and processed using Autobacend software (S.L., unpublished). All trace files were submitted to the NCBI trace archive (http://www.ncbi.nlm.nih.gov/Traces).

Map construction

Fingerprints were assembled into contigs using FPC[12] with a Sulston score threshold of 1×10^{-17} and tolerance of 7. CORAL was used to improve clone order after automated contig builds using FPC. During the manual review of clone order, fingerprint band patterns were compared across a contig. A given band should be found in all correctly positioned clones from a limited region, allowing one to scan a contig from one end to the other, confirming the presence or absence of bands in adjacent clones. An incorrectly positioned clone breaks the overall continuity of banding pattern and was repositioned or removed from the contig. Occasionally, a lane had the fingerprint patterns of two unrelated clones. Clone contamination was confirmed by searching the FPC database for the clone with a similar library name and an exact subset of bands. In this way 576 contaminated clones were removed. A limited number of partially digested clones were also removed from contigs. It is particularly important to order correctly the clones at contig ends. Overlapping contigs are identified by comparing the fingerprints of clones at their ends using successively relaxed Sulston score thresholds. Clones are added from the singleton pool as needed to increase coverage in junction regions when contigs are merged. No attempt was made to add all singleton clones to contigs.

Addition of white leghorn clones

White leghorn clones did not integrate well into contigs made from the larger RJF clones owing to small insert sizes. The white leghorn clones tended to form separate contigs,

Table 2 **Summary statistics of chicken clone map coverage by chromosome**

Chicken chromosome	Chromosome size (cM)	Sequenced chromosome size (Mb)	Total fingerprint contigs	Fingerprint contig coverage (Mb)
GGA1	553	188.2	29	188.8
GGA2	474	147.6	18	146.9
GGA3	317	108.6	19	107.8
GGA4	270	90.6	8	88.7
GGA5	199	56.3	10	53.1
GGA6	116	33.9	2	34.1
GGA7	165	37.3	5	32.6
GGA8	105	30	3	26.1
GGA9	132	23.4	6	23.8
GGA10	120	20.9	4	19.7
GGA11	90	19	3	20.0
GGA12	90	19.8	5	18.1
GGA13	74	17.3	4	18.1
GGA14	77	20.6	8	18.1
GGA15	60	12.4	3	12.4
GGA16	60	0.2	2	1.1
GGA17	70	19.6	4	10.1
GGA18	48	8.9	3	8.9
GGA19	41	9.5	3	10.0
GGA20	62	13.5	4	13.7
GGA21	70	6.2	3	6.0
GGA22	21	2.2	3	3.2
GGA23	11	5.7	3	5.3
GGA24	60	5.9	3	5.8
GGA26	54	4.3	2	4.0
GGA27	60	2.7	5	3.5
GGA28	74	4.7	8	6.1
GGA32	21	1	1	0.4
GGAW	NE	0.15	5	2.8
GGAZ	100	31	45	60.8
E22C19W28	NE	0.07	1	0.5
E26C13	52	0.23	2	0.7
E50C23	41	0.02	2	0.7
Unlocalized	NE	121	34	13.1
Total	3,687	1,063	260	964.9

NE, not estimated.

largely because the Sulston score takes into account total band number as well as the number of bands matching between two clones. Consequently, white leghorn clones were added to the map based only on their fingerprint matches to RJF clones. The criteria for adding white leghorn clones to RJF contigs were Sulston score threshold and number of RJF contig clones matching at that threshold. We examined the number of marker position inconsistencies introduced after adding white leghorn clones to choose appropriate parameters. The heuristically determined requirement of fingerprint matches to a minimum of five RJF contig clones at a Sulston score threshold of 1×10^{-6} limited marker inconsistencies yet added a significant number of white leghorn clones with positional information to contigs. Manual comparisons of white leghorn fingerprints to surrounding clones were limited to the clones that have assigned STS markers.

Fingerprint map linkage to the draft sequence assembly

The threshold of BES links used to declare regions of the fingerprint map and draft sequence collinear is subjective. Our choice of parameters was guided by their impact on the frequency of marker inconsistencies and number of topologically impossible contig combinations. A minimum of six BES links were required before aligning an FPC contig to a sequenced region, provided that 70% of all links were consistent and no topological constraints were violated. With these parameters, the overall frequency of spurious BES links from an FPC contig to non-collinear sequence contigs was 0.10 and the frequency of BES links to sequence contigs that were not aligned to the FPC map was 0.05. Discrepant BES links reveal regions of the FPC map where manual review might be useful. Each FPC contig was scanned using a sliding window of ten BES links, which corresponds to roughly 109 kb (assuming a genome size of 1.06 Gb and a random distribution of BES links). If four or more BES links were to an unaligned sequence contig, that FPC region was flagged for review. Of the 195 flagged regions, 14 contained contaminated clones. The remaining number could be a reflection of the reliability of BES links, errors in fingerprint map not revealed by fingerprints, or errors in the sequence assembly.

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Early blastomere determines embryo proliferation and caste fate in a polyembryonic wasp

Vladimir Zhurov*, **Tomislav Terzin*** & **Miodrag Grbić**

Department of Biology, The University of Western Ontario, London N6A 5B7, Canada

* These authors contributed equally to this work.

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Polyembryonic development is a unique mode of metazoan development in which a single zygote generates multiple embryos by clonal proliferation[1]. The polyembryonic parasitic insect *Copidosoma floridanum* shows one of the most extreme cases of polyembryony, producing up to 2,000 embryos from a single egg. In addition, this wasp exhibits an unusual polyphenism, producing two morphologically distinct larval castes, termed precocious and reproductive, that develop clonally from the same zygote[2]. This form of development seems incompatible with a model of insect development in which maternal pre-patterning of the egg specifies embryonic axial polarity[3]. Here we show that maternal pre-patterning in the form of germ plasm creates cellular asymmetry at the four-cell stage embryo of *Copidosoma* that is perpetuated throughout development. Laser ablations of cells show that the cell inheriting the germ plasm regulates both the fate and proliferation of the reproductive caste. Thus, we have uncovered a new mechanism of caste specification, mediated by the regulatory capacity of a single cell. This study shows that the evolution of mammalian-like regulative development of an insect embryo relies on a novel cellular context that might ultimately enhance developmental plasticity.

Polyphenism is a phylogenetically widespread feature of some plants and animals, in which organisms with complex life histories form different phenotypes from a single genome[4]. Because model organisms do not exhibit polyphenism, the developmental basis of this marked genetic plasticity remains largely undetermined. Traditional polyphenic eusocial species such as ants and bees produce reproductive (queen) and sterile (worker) castes from the same genome when separate zygotes are exposed to different hormonal or environmental influences[5]. However, one of the most extreme examples of polyphenism coupled with a complex life history strategy is the clonal production of two different morphs from the same zygote in the polyembryonic parasitic wasp *Copidosoma floridanum*.

Copidosoma parasitizes eggs of the moth *Trichoplusia ni* (Fig. 1a). In contrast with other insects that undergo syncytial early cleavages in which nuclear divisions do not follow the division of cytoplasm[6], the *Copidosoma* egg undergoes total cleavage forming individual cells[7]. During the first two days, the wasp embryo emerges from its chorion and forms a primary morula surrounded by the extra-embryonic membrane derived from the polar body region (Fig. 1a, grey)[8]. The primary morula then undergoes a series of embryonic movements to 'implant' itself into the developing host embryo (Supplementary movie). After parasitization, the host larva emerges and undergoes five larval instars. During the first to third host larval instars, *Copidosoma* enters the proliferative phase generating many proliferative morulae, each containing thousands of round and apparently non-differentiated cells[8]. This phase represents the developmental novelty initiating the clonal production of up to 2,000 embryos. In the proliferative phase the developmental programme bifurcates to produce two terminal larval phenotypes. During the first to third larval instars some of the morulae initiate