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POLYCHLORINATED BIPHENYL CONTAMINATION AND MINISATELLITE DNA MUTATION RATES OF TREE SWALLOWS

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Abstract—The evidence that exposure to polychlorinated biphenyls (PCBs) leads to mutations is equivocal and controversial. Using multilocus DNA fingerprinting, we compared the mutation rate of tree swallows (*Tachycineta bicolor*) nesting at sites with high and low levels of contamination with PCBs. The upper Hudson River, USA, is highly contaminated with PCBs as a result of releases from two capacitor manufacturing plants in Hudson Falls and Fort Edward, New York, USA. Tree swallows nesting nearby have some of the highest known concentrations of PCBs in their tissues of any contemporary bird population (up to 114,000 ng PCB/g tissue). We found no difference in mutation rates between sites in New York with high PCB contamination and reference sites in Wisconsin, USA, and Ontario and Alberta, Canada, with known or presumably low levels of contamination. Thus, the mechanism behind altered reproductive behavior of tree swallows along the upper Hudson River is most likely physiological impairment, such as endocrine disruption, rather than mutation.

Keywords—Hudson River Mutation rate Tree swallow Polychlorinated biphenyls

INTRODUCTION

Recent studies have shown that chemicals and radiation in the environment are associated with higher rates of deoxyribonucleic acid (DNA) mutation in vertebrates. For example, acute exposure to radiation following the Chornobyl nuclear accident was correlated with higher mutation rates of humans [1] and barn swallows (*Hirundo rustica*) [2]. Mutation rates are also higher at sites contaminated by industrial pollution. Mutation rates of herring gulls (*Larus argentatus*) in the heavily polluted harbor of Hamilton (ON, Canada) were almost three times greater than those of less polluted locations [3]. Winter flounder (*Pseudopleuronectes americanus*) [4] in Boston Harbor and Atlantic tomcod (*Microgadus tomcod*) [5] in the Hudson River also showed genetic changes that were not found in populations from less polluted locations. Although it is well known that certain chemicals can induce mutations in laboratory experiments, it is less clear if these chemicals also lead to heritable mutations in the offspring of wild organisms. Few studies have examined mutation rates in wild populations because mutations are very rare events and, until recently, efficient assays were not available to estimate their frequency. Despite their rarity, small heritable mutations may accumulate and result in increased genetic disease [6]. Thus, they may provide an early warning of more subtle and longer-term environmental problems.

Polychlorinated biphenyls (PCBs) are known to be toxic through their binding to the aryl hydrocarbon (Ah) receptor and subsequent induction of metabolites (electrophiles and alkylating agents) [7]. The evidence that PCB exposure also leads to mutations is equivocal and highly controversial [7–9]. Polychlorinated biphenyls or their metabolites may cause DNA damage directly [8] or by forming adducts that may lead to DNA damage [10,11]. Recently, a laboratory study of mice by Hedenskog et al. [12] examined the effects of PCB exposure on rates of mutation in minisatellite DNA. Their results showed that exposure to PCBs induced a significant increase in mutation rate at one locus (PC-1) but not at another (PC-2). This suggests that the mutagenic effects of PCBs are variable. Thus, studies surveying more loci may provide a more complete understanding of the mutagenic effects of PCBs.

Here we report the use of multilocus DNA fingerprinting to examine mutation rates of tree swallows (*Tachycineta bicolor*) nesting in the upper Hudson River valley of New York State, USA, at sites with high levels of contamination from PCBs. Tree swallows feed on aquatic insects that emerge from river sediments, and, in the upper Hudson River, these aquatic insects have PCB levels correlated with levels found in nearby sediments of the river [13]. The distribution of PCB congeners in the tissue of swallows is similar to that of congeners in the sediment and benthic insects. Nestlings fed insects emerging from the Hudson River accumulate PCBs, demonstrating that the PCBs in tree swallow tissues come from the Hudson River [13]. Mean PCB levels in tree swallow nestlings at the two Hudson River valley sites in 1994 and 1995 ranged from 39,800 to 62,200 ng/g ,and a single adult sampled from one of the sites had 114,000 ng PCB/g [13]. These are some of the highest known body concentrations of PCBs in contemporary bird populations [13]. Thus, these birds provide an excellent opportunity to test the hypothesis that PCBs increase the rate of mutations in wild populations.

METHODS

Study sites and fieldwork

Tree swallows were studied in 1998 at two contaminated locations in New York (Remnant 4 and Special Area 13) along the upper Hudson River near the town of Hudson Falls (43°18′N, 73°29′W; see fig. 1 in [13]). At each site, 25 to 35 nest boxes were placed within 100 m of the river's edge. The

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Table 1. Mean concentrations (ng/g) of total polychlorinated biphenyls in tissue of nestling tree swallows along the Upper Hudson River and from the reference site in Saukville (WI, USA) in 1998. Data from previous studies provided for comparison

Location	ng/g	Reference
Upper Hudson River, New York, USA		
Remnant Deposit 4	7,900	This study
Special Area 13	9,950	
Saukville (WI, USA)	49	This study
Lower Fox River (WI, USA)	2,500	[38]
Lake Huron (ON, Canada)	10	[39]
Lake Ontario (ON, Canada)	750	[39]
Saginaw River (MI, USA)	1,000	[40]

1998 reference site was at the University of Wisconsin–Milwaukee Field Station (Saukville, WI, USA; 43°23′N, 88°01′W). We also included reference site data from our previous DNA fingerprinting studies of populations of tree swallows in Ontario (44°34′N, 76°20′W) and Alberta (53°38′N, 112°36′W) in 1990 and 1991 [14–16]. Chemical analyses of tree swallows in other areas have indicated much lower levels of PCB contamination than along the upper Hudson River (Table 1; see also fig. 3 in [13]). None of the reference sites was near industrial activity, and thus we presumed these sites were less contaminated than the New York sites. In the case of the Wisconsin site, we provide data here to support this assumption. All nests were checked every 2 d to monitor stage of reproduction. Adults were captured in the nest boxes.

PCB analyses

Polychlorinated biphenyl concentration in tree swallow nestlings (14 d old) was estimated at the two Hudson River sites and in Wisconsin in 1998, similar to our previous studies [13]. For PCB analysis we collected two nestlings from each of four nests at each of the two Hudson River study sites (16 nestlings) and one nestling from each of two nests in Wisconsin (two nestlings). Nestlings were euthanized using carbon dioxide and approved techniques ([17]; see Acknowledgement for permits). All nestling samples were stored in chemically clean containers and kept frozen until analysis. Polychlorinated biphenyl congener analysis of tree swallow nestlings was performed by the Columbia Environmental Research Center (Biological Resource Division, U.S. Geological Survey, Columbia, MO) using capillary gas chromatography/electron capture detection. All gas chromatography/electron capture detection analyses were performed using a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with cool on-column capillary injection systems. Total PCB concentrations for these samples were determined by summing the concentrations of the PCB congeners [18].

DNA fingerprinting

Our DNA fingerprinting techniques are described in detail by [14,15]. Small (50-μl) blood samples were taken from all members of each family. Blood was stored in lysis buffer [19] at 4°C until the DNA was extracted using a 5-M salt solution [20]. After digestion with *Hae* III, DNA was subjected to electrophoresis (8 μg of DNA per lane) for approximately 48 h and Southern blotted onto Hybond N+ (Amersham, Piscataway, NJ, USA) transfer membranes. Minisatellite probes were radioactively labeled by primer extension, added to 20 ml of hybridization solution [21], and allowed to hybridize at 65°C with the membranes overnight. After washing the membranes, we exposed them to x-ray film for 1 to 7 d to produce the autoradiographs. All membranes were hybridized with the *per* probe [22]. We subsequently reprobed some membranes with Jeffreys's 33.15 [23] or M13 [24] when nestlings had relatively few bands (10–15 bands), or nestlings were close to our criteria for exclusion of parents (see the following discussion).

Scoring and analysis of DNA fingerprints

The *per* probe produces multilocus profiles (fingerprints) consisting of at least 10 bands or fragments for each individual in the 2- to 24-kb range [14]. In this study we examined 10 to 29 bands per individual with the *per* probe (mean ± standard deviation [SD] = 16.5 ± 4.4). In a previous study of tree swallows [14], unrelated individuals shared 0.248 (±0.012 SD) of their bands (range = 0.01.86–0.325). Using this value (0.248) and the average number of bands scored per individual (16.5), the probability that any two individuals would have the same fingerprint profile was $0.248^{16.5}$, or less than 10^{-9}. Mutation rate was estimated from the number of DNA fragments in the fingerprint profile of nestlings that did not match any of the fragments in the profiles of putative parents [14]. These unattributable or novel fragments could occur because the nestling is not related to one or both parents or because of mutation. Related nestlings that are direct descendants of the parents typically have no novel fragments or, in a small percentage of individuals, just one or two novel fragments that arise by mutation. In previous studies of tree swallows, the probability that an individual would have two novel fragments was 0.072 and 0.011 for three novel fragments [16]. Related nestlings also share at least 50% of their bands on average with each parent, as expected by Mendelian inheritance. In contrast, some nestlings have a considerable number of novel fragments (>2) and share few of their fragments with their putative father (although they almost always share a high proportion with their mother). These extrapair young are the result of copulations between females on one territory and males on another, and thus the young are not the direct descendants of the putative father. As a consequence, the large numbers of novel fragments in these nestlings are inherited from another (extrapair) male and are not due to mutation. Extrapair young are common in tree swallows, varying from 38% to 76% of young [16,25] and could not be used for estimates of mutation rate.

We identified extrapair young using both the number of novel fragments and band sharing between nestlings and each putative parent. Based on our previous studies [14–16], we excluded nestlings as direct descendants of one or both putative parents when they had three or more novel fragments with the *per* probe and a low proportion of bands shared between the nestling and a parent (<0.40; see also Fig. 1). Band sharing was estimated as two times the number of shared bands between a nestling and putative parent divided by the total number of bands scored in the nestling and parent [26]. All of our excluded nestlings shared more bands with their putative mother (all >0.40) than their putative father (see Results). Therefore, we concluded that all of our unrelated nestlings were the result of extrapair copulation rather than conspecific egg parasitism (egg dumping).

Statistical analysis

Mutation rates are usually on the order of one to three per thousand DNA fragments scored [27], although sites with high

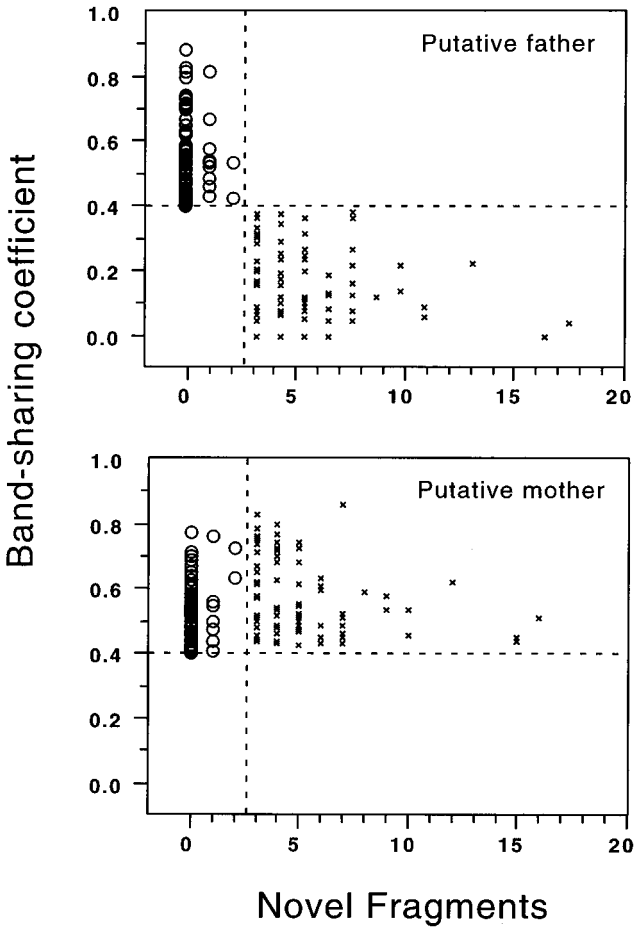

Fig. 1. Band-sharing coefficients and novel fragments of each offspring with their putative father (top panel) or mother (bottom panel). Data are from 178 nestlings sampled along the upper Hudson River, New York, USA. Circles indicate within-pair young (n = 89), and x's indicate extrapair young (n = 89). Dashed lines indicate our criteria for excluding young as direct descendants (band sharing < 0.40 and > 2 novel fragments). See Methods for more details.

mutation rates approach 20 per thousand [3]. Mutation rate was calculated for each location as the number of novel fragments from all within-pair nestlings divided by the total number of bands scored (all probes combined). In one case, a single mutation in a nestling from Wisconsin was detected by both probes. This was considered a single mutation event; thus, only the values for one of the probes (*per*) were used in the case of this nestling. Differences among sites in mutation rate were examined with a likelihood chi-square test of independence.

RESULTS

In our analysis of 178 nestlings from New York and 35 nestlings from Wisconsin, we excluded 102 extrapair offspring from our analysis. These nestlings had three or more novel fragments and low band sharing (<0.40) with the putative father but not the putative mother (Fig. 1). Of the remaining 89 nestlings from New York, 77 nestlings had no novel fragments, 10 nestlings had one novel fragment, and two nestlings had two novel fragments (Fig. 1). Among these 89 within-pair young from New York, the lower 95% confidence interval (CI; one-tailed) for band sharing with their parents was 0.379, similar to the cutoff (0.400) used in previous studies [14]. Our results would not change significantly if we used 0.379 as the cutoff for relatedness instead of 0.400, as only one borderline nestling was present (band sharing with male = 0.380), and including its novel fragments (n = 7) changed the mutation rate slightly (from 0.009 to 0.012; see Table 2). In Wisconsin, 22 nestlings were considered direct descendants of the putative parents, as they had few novel fragments (<3) and high (>0.40) band-sharing coefficients. Within-pair nestlings from Ontario (n = 109) and Alberta (n = 26) were identified using the same criteria as in this study [14–16].

We identified 18 novel bands in 89 within-pair nestlings from contaminated sites in New York. At our reference sites, 50 novel bands were detected in 157 within-pair nestlings (Table 2). Mutation rates were similar in the combined reference (0.011 per meiotic event) and contaminated (0.009) sites (G = 0.82, df= 1, $p > 0.36$). Analyzing each site separately also revealed no significant difference in mutation rate (G = 1.1, df = 4, $p > 0.89$).

Total PCB concentration in 1998 ranged from 7,600 to 12,000 ng/g (wet mass) of body tissue in nestlings at the two Hudson River sites (mean ± SD of eight composite samples = 8,913 ± 1,495 ng/g). In Wisconsin two nestlings had total PCB concentrations of 42 and 56 ng/g of body tissue, verifying the low level of PCB at this reference site.

DISCUSSION

We found no difference in minisatellite mutation rate of swallows breeding in areas of relatively high and low PCB contamination. Mutation rates of minisatellite DNA are usually on the order of 1 to 10 per thousand DNA fragments scored [27,28] similar to the rate observed in this study (10 per thousand). In contaminated animals, mutation rates approach 20

Table 2. Minisatellite DNA mutation rates in tree swallow nestlings using all probes (see Methods)

Location	No. nestlings scored	No. mutations	No. fragments scored	Mutation rate per fragment scored
Contaminated sites				
Remnant 4, New York, USA	47	10	1,076	0.010
SA13, New York, USA	42	8	928	0.010
Combined	89	18	2,004	0.009
Reference sites				
Wisconsin, USA	22	7	584	0.012
Alberta, Canada	26	5	538	0.009
Ontario, Canada	109	38	3,243	0.012
Combined	157	50	4,365	0.011

per thousand fragments [3]. If such a high mutation rate occurred in tree swallows, then we should have found 37 novel fragments in the more contaminated sites (based on the number of fragments scored); however, we found just 18 novel fragments. Our pooled sample sizes (including all reference sites) were sufficient to detect a difference in mutation rate of about 0.5 times, which is smaller than the differences reported elsewhere. For example, the mutation rate of herring gulls was two times higher in contaminated than reference sites [3], and mutation rates of barn swallows from Chornobyl were 2 to 10 times higher than birds from reference sites [2].

Our results contrast with a recent study that found a higher rate of mutation in laboratory mice injected with PCB (Aroclor 1254) [12]. Hedenskog et al. [12] found six mutations among 51 alleles in their PCB treatment group (0.118), which was significantly greater than the control group (0 mutations among 43 alleles). However, in their study, mutations were found at only one of the two loci examined, suggesting that if PCBs do indeed cause mutations, the effect is variable. Hedenskog et al. [12] suggested that Aroclor 1254 was genotoxic because it interfered with recombination. Similarly, germ-line mutations in human minisatellites are thought to occur as a consequence of unequal sister chromatid exchange, gene conversions, or replication slippage [29,30].

A number of explanations exist for why our results differ from those of Hedenskog et al. [12]. First, differences may have existed in exposure to PCBs. The mice studied by Hedenskog et al. [12] had at least 10 times the concentration of PCBs observed in tree swallows. In this study the highest mean level of contamination in tree swallow nestlings was 12,000 ng total PCB/g body mass, while Hedenskog et al. [12] injected mice twice with 100,000 ng Aroclor 1254/g body mass over a period of approximately 12 d. The route of exposure also differed, as mice were injected with PCBs while swallows were exposed through their diet. Also, the PCB congener composition may have differed between studies. Substantial variability exists in the toxicological mode of action and the severity of effects among PCB congeners [31]. Lastly, it is well known that the effects of PCB contamination vary among species [31]. Even different strains of laboratory mice differ in mutation rate for the same minisatellite locus [12]. In birds, for example, PCB contamination has a wide range of physiological effects on reproduction and behavior, depending on the species [32,33]. In fact, it appears that tree swallows are able to tolerate higher levels of PCB exposure than other species. Tree swallows nesting along the upper Hudson River valley do not appear to have some of the extreme morphological deformities seen in other contaminated species [34], although they do have abnormal plumage development [35]. Additionally, tree swallows in this population have higher rates of nest abandonment [36] and make poorer-quality nests [37].

In summary, we found no effect of PCB contamination on the mutation rate of minisatellite DNA of tree swallows. It is apparent that DNA fingerprinting is a useful technique for testing effects of contaminants on wildlife, as minisatellite DNA has relatively high rates of mutation, and many loci (at least 10 in this study) can be surveyed simultaneously, thus reducing the need for large sample sizes. Contrasting results from different studies suggest the effects of PCB contamination are complex. Further studies are necessary to elucidate the effects of high concentrations of PCBs on mutation rates in both laboratory and wild vertebrates.

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REFERENCES

1. Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ. 1996. Human minisatellite mutation rate after the Chernobyl accident. *Nature* 380:683–686.
2. Ellegren H, Lindgren G, Primmer CR, Møller AP. 1997. Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature* 389:593–596.
3. Yauk CL, Quinn JS. 1996. Multilocus DNA fingerprinting reveals high rate of heritable genetic mutation in herring gulls nesting in an industrialized urban site. *Proc Natl Acad Sci USA* 93:12137–12141.
4. McMahon G, Huber LJ, Moore MJ, Stegman JJ, Wogan GN. 1990. Mutations in c-Ki-*ras* oncogenes in diseased livers of winter flounder from Boston Harbor. *Proc Natl Acad Sci USA* 87: 841–845.
5. Wirgin I, Kreamer GL, Garte SJ. 1991. Genetic polymorphism of cytochrome P-450IA in cancer prone Hudson River tomcod. *Aquat Toxicol* 19:205–214.
6. Crow JF. 1997. The high spontaneous mutation rate: Is it a health risk? *Proc Natl Acad Sci USA* 94:8380–8386.
7. Safe S. 1989. Polychlorinated biphenyls (PCBs): Mutagenicity and carcinogenicity. *Mutat Res* 220:31–47.
8. Dobson S, van Esch GJ. 1993. *Polychlorinated Biphenyls and Terphenyls.* Environmental Health Criteria Series, 2nd ed. World Health Organization, Geneva, Switzerland.
9. U.S. Environmental Protection Agency. 1996. PCBs: Cancer dose–response assessment and application to environmental mixtures. Office of Research and Development, Washington, DC.
10. Dubois M, Pfohl-Leszkowicz A, Grosse Y, Kremers P. 1995. DNA adducts and P450 induction in human, rat and avian liver cells after exposure to polychlorobiphenyls. *Mutat Res* 345:181–190.
11. McLean MR, Robertson LW, Gupta RC. 1996. Detection of PCB adducts by the ^{32}P-postlabeling technique. *Chem Res Toxicol* 9: 165–171.
12. Hedenskog M, Sjogern M, Cederberg H, Rannug U. 1997. Induction of germline-length mutations at the minisatellites PC-1 and PC-2 in male mice exposed to polychlorinated biphenyls and diesel exhaust emissions. *Environ Mol Mutagen* 30:254–259.
13. Secord AL, McCarty JP, Echols K, Meadows JC, Gale RW, Tillitt DE. 1999. Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in tree swallows from the upper Hudson River, New York State, USA. *Environ Toxicol Chem* 18: 2519–2525.
14. Lifjeld JT, Dunn PO, Robertson RJ, Boag PT. 1993. Extra-pair paternity in monogamous tree swallows. *Anim Behav* 45:213–229.
15. Dunn PO, Whittingham LA, Lifjeld JT, Robertson RJ, Boag PT. 1994. Effects of breeding density, synchrony, and experience on extrapair paternity in tree swallows. *Behav Ecol* 5:123–129.
16. Dunn PO, Roberston RJ, Michaud-Freeman D, Boag PT. 1994. Extra-pair paternity in tree swallows: Why do females mate with more than one male? *Behav Ecol Sociobiol* 35:273–281.
17. American Ornithologists' Union. 1998. Report of the committee on the use of wild birds in research. *Auk* 105(Suppl. A):1–41.
18. Schwartz TT, Stalling DL. 1991. Chemometric comparison of polychlorobiphenyl residues and toxicologically active polychlorobiphenyl congeners in the eggs of Forster's tern (*Sterna fosteri*). *Arch Environ Contam Toxicol* 20:183–199.
19. Seutin G, White BN, Boag PT. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69:82–90.
20. Miller S, Dykes D, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215.

21. Westneat DF, Noon WA, Reeve HK, Aquadro CF. 1988. Improved hybridization conditions for DNA ''fingerprints'' probed with M13. *Nucleic Acids Res* 16:4161.
22. Shin HS, Bargiello TA, Clark BT, Jackson FR, Young MW. 1985. An unusual coding sequence from a *Drosophila* clock gene is conserved in vertebrates. *Nature* 317:445–448.
23. Jeffreys AJ, Wilson V, Thein SL. 1985. Individual-specific ''fingerprints'' of human DNA. *Nature* 316:76–79.
24. Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AS, Christophe D. 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. *Science* 235: 683–684.
25. Barber CA, Robertson RJ, Boag PT. 1996. The high frequency of extra-pair paternity in tree swallow is not an artifact of nestboxes. *Behav Ecol Sociobiol* 38:425–430.
26. Wetton JH, Carter RE, Parkin DT, Walters D. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327:147–149.
27. Jeffreys AJ, Royle N, Wilson V, Wong Z. 1988. Spontaneous mutation rates to new length alleles at tandem repetitive hypervariable loci in human DNA. *Nature* 332:278–281.
28. Westneat DF. 1990. Genetic parentage in the indigo bunting: A study using DNA fingerprinting. *Behav Ecol Sociobiol* 27:67–76.
29. Jeffreys AJ, Tamaki K, MacLeod A, Monckton DG, Armour JAL. 1994. Complex gene conversion events in germline mutation at human minisatellites. *Nat Genet* 6:136–145.
30. Buard J, Vergnaud G. 1994. Complex recombination events at the hypermutable minisatellite CEB1 (D2S90). *EMBO J* 13:3203–3210.
31. Safe S. 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* 24:87–149.
32. Hoffman DJ, Rice CP, Kubiak TJ. 1996. PCBs and dioxins in birds. In Berterm WN, Heinz GH, Redmon-Norwood AW, eds, *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations.* CRC, New York, NY, USA, pp 165–207.
33. Barron MG, Galbraith H, Beltman D. 1995. Comparative reproductive and developmental toxicology of PCBs in birds. *Comp Biochem Physiol C* 112:1–14.
34. Hoffman DJ, Melancon MJ, Klein PN, Eisemann JD, Spann JW. 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels, and common terns. *Environ Toxicol Chem* 17:747–757.
35. McCarty JP, Secord AL. 2000. Possible effects of PCB contamination on female plumage color and reproductive success in Hudson River tree swallows. *Auk* 117:991–999.
36. McCarty JP, Secord AL. 1999a. Reproductive ecology of tree swallows (*Tachycineta bicolor*) with high levels of polychlorinated biphenyl contamination. *Environ Toxicol Chem* 18:1433–1439.
37. McCarty LP, Secord AL. 1999b. Nest-building behavior in PCB-contaminated Tree Swallows. *Auk* 116:55–63.
38. Ankley GT, et al. 1993. Uptake of planar polychlorinated biphenyls and 2,3,7,8-substituted polychlorinated bibenzofurans and dibenzo-*p*-dioxins by birds nesting in lower Fox River and Green Bay, Wisconsin, USA. *Arch Environ Contam Toxicol* 24:332–344.
39. Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K. 1995. Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds, and tree swallows from wetlands in the Great Lakes-St. Lawrence River basin. *Environ Toxicol Chem* 14:491–501.
40. Nichols JW, Larsen CP, McDonald ME, Niemi GJ, Ankley GT. 1995. A bioenergetics-based model for accumulation of polychlorinated biphenyls by nesting tree swallows, *Tachycineta bicolor*. *Environ Sci Technol* 29:604–612.