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Editor
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ISOLATION, IDENTIFICATION AND ANTIBIOTIC RESISTANCE OF GRAM NEGATIVE BACTERIA FROM ROSS COMMON CREEK, SAYLORSBURG, PENNSYLVANIA[1]

HARUN GULTEKIN and JANE E. HUFFMAN[2]

Department of Biological Sciences, Fish & Wildlife Microbiology Laboratory, East Stroudsburg University, East Stroudsburg, PA 18301

ABSTRACT

Gram-negative bacteria assemblages and their antibiotic resistance were determined from three sites along Ross Common Creek, Monroe County, in Saylorsburg, Pennsylvania. Twelve bacterial species were isolated. The genera isolated included *Escherichia*, *Klebsiella*, *Alcaligenes*, *Citrobacter*, *Pseudomonas*, *Pantoea*, *Serratia*, and *Aeromonas*. Reservoirs of antibiotic resistance did not differ between the three sites. Bacteria exhibiting tetracycline resistance were examined for the presence of plasmids. Plasmids were detected in *Klebsiella* and *E. coli*. High copy of DNA occurred in one of the *E. coli* isolates. The sizes of the plasmids isolated ranged from 3,000bp–10,000 bp.
[J PA Acad Sci 82(1): 3–6, 2008]

INTRODUCTION

Streams and rivers are major links between terrestrial environments and oceans, and bacteria play an important role in lotic environment (Leff 1994). The presence of both potentially pathogenic Gram-negative bacteria and fecal coliforms in waterways that receive agricultural runoff raises the question of whether resistance transfer may occur in streams, rivers, bays and other waterways (Kelch and Lee 1978). Population and diversity of stream organisms are controlled by a variety of biotic and abiotic factors. Isett and Huffman (2001) provided information on how agricultural land-use practices influence bacterial diversity in a stream ecosystem. Myers and Huffman (2001) provided information on temporal and spatial scales in the bacterial assemblages in Broadheads Creek. Bacteria possess unique adaptive features, which allow them to adapt to changes in the environment (Lobova et al. 2002). Goni-Urriza et al. (2000) evaluated the impact of an urban effluent on antibiotic resistance of the bacterial populations isolated.

The widespread use of antibiotics has led to the emergence of resistant strains of bacteria (Levy 1978). The increased use of antimicrobials in farming, together with the practice of raw sewage discharge into receiving waters, has resulted in a significant increase in the numbers of antibiotic resistant bacteria present in aquatic environments (Young 1993). During the last decade there has been a decline in the effectiveness of antibiotics. Many genes conferring antibiotic resistance are located on mobile genetic elements (e.g., plasmids, transposons and integrons). Preliminary studies about antibiotic resistance in bacteria indicate that the frequency of antibiotic resistance is significantly elevated in heavy metal-contaminated environments, including stream sediments, benthic fish, and organic foam (McArthur and Tuckfield 2000).

This study was undertaken to provide information on how agricultural land use practices influence bacterial diversity in Ross Common Creek. To address this, Gram-negative bacterial assemblages were determined using temporal and spatial scales. The bacteria were also screened for the resistance to antibiotics, and the presence of plasmids.

MATERIALS AND METHODS

The Study Stream

Ross Common Creek (40.865°N; 75.304°W), is located in Monroe County, Pennsylvania. The creek originates in Wind Gap, and continues through Saylorsburg to Palmerton. The creek is spring fed. The creek flows in a southeast direction and enters a lake. The creek flows through forest habitat, residential area, a camp, mulch factory, and a dairy farm. Three sample sites were selected along the course of the creek for sample collection. Site 1 was located 100 feet downstream from the reservoir, site 2 was approximately 2 miles downstream from site 1, and site 3 was at Saylor Lake which is fed by Ross Common Creek. Samples were collected biweekly from June 30, 2003 to August 30, 2003.

Sample Collection and Bacterial Isolation

The samples were collected 10 cm below the surface of the water using sterile 500 ml capped bottles. Water temperature was taken at the time of sampling at 10 cm below the water surface. pH was also measured. Water samples were processed within 2 hours of collection. A 10 ml and 100 ml sample was filtered through a 0.45*um* Millipore filter (Bed-

[1]Submitted for publication 29 December 2004; accepted 8 February 2008.
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ford, MA 01730). The filters were placed on MacConkey agar (MAC) and incubated at 37°C for 24 hours. Isolated bacterial colonies were then cultured on Trypticase Soy Agar (TSA) and MAC.

Bacterial Identification and Antibiotic Sensitivity Testing

Isolated colonies from MAC were diluted in 5 ml of sterile distilled water and 2 ml was then pipetted onto Analytical Profile Index 20E (bioMerieux Vitek, Inc., Durham, NC) strips for biochemical identification. After identification of the bacteria the isolates were tested for reservoirs of antibiotic resistant (Kirby Bauer Technique) using penicillin G (10 IU), oxacillin (1*ug*), amoxillin and clavanic acid (20/10 *ug*), kanamycin (30*ug*), streptomycin (10*ug*), cephalothin (30*ug*), chloramphenicol (30*ug*), tetracycline (30*ug*), oxytetracycline (30*ug*), cefotaxime (30*ug*), ciprofloxacin (5*ug*), ceftazidime (30*ug*), and imipenem (10*ug*) (Becton Dickinson, Cockeysville, MD).

Plasmid Extraction

Bacteria that were resistant to the antibiotics tested were grown in 5 ml of trypticase soy broth overnight at 37C with shaking. The Qiagen Plasmid Mini kit (Qiagen Sciences, Valencia, CA 91355) was used for extraction of plasmid DNA from the bacterial cells. Plasmids were run on 1% agarose gels to determine size.

RESULTS

Gram negative bacteria were isolated from all three sites in Ross Common Creek. The pH values ranged from 5.6 to 6.7. The temperature ranged from 15–23°C from June to August. The species of bacteria isolated from the three sites are summarized in Table 1. The number of bacterial species was greater at site 3 (9 species) followed by site 2 (8 species) and site 1 (7 species). All three sites had the following isolates in common: *Escherichia coli*, *Klebsiella oxytoca*, *Alcaligenes* sp., *Citrobacter freundii*, and *Aeromonas hydrophila*. *Pantoea agglomerans* was isolated only at site 1. *Klebsiella pneumoniae* was isolated only at site 2. The number of bacterial species isolated increased temporally at the three sites. The number of species isolated in June was 7, followed by 8 in July, and 9 in August (Table 2). *Pantoea agglomerans* and *Pseudomonas stutzeri* were isolated only in June. *Pseudomonas aeruginosa* was isolated only in July, and *Klebsiella pneumoniae* only in August. All of the isolated bacteria were resistant to penicillin, oxacillin, methicillin and ampicillin. Tetracycline, oxytetracycline and cephalothin resistance occurred in *Serratia* sp. and *Serratia marcescens*. *Pseudomonas* sp. and *Pantoea agglomerans* were resistant to chloramphenicol. All of the isolated bacteria were susceptible to cefotaxime, ceftazidime and ciprofloxacin. *Alcaligenes* sp. isolates from sites 1 and 2 were resistant to imipenem while the isolates from site 3 were susceptible. All resistant bacteria were checked for the presence of plasmids. Plasmids were detected in *Klebsiella* and *E. coli*. High copy of DNA occurred in one of the *E. coli* isolates. The sizes of the plasmids isolated ranged from 3,000 bp–10,000 bp.

DISCUSSION

The bacterial assemblages identified by Isett and Huffman (2001), and Myers and Huffman (2001), were similar to those reported in this study. These researchers reported that seasonal changes may influence the type of bacterial assemblages isolated. In this study, species diversity between sites did not differ greatly, this may be the result of sampling being done only in the summer months. The bacteria species isolated in this study have been reported to occur in streams and rivers (Atlas, 1997). *Aeromonas hydrophila*, isolated from all three sites in July and August, has importance both economically and medically. Members of this genus are distributed in freshwater and sewage, as well as in association with aquatic animals (Cahill 1990, Stecchini and Domenis 1994). *Aeromonas* sp. are known to cause a diverse spectrum of diseases in both warm and cold-blooded animals (Trust 1986, Janda 1991).

Table 1. Gram-negative bacteria identified from three sites on Ross Common Creek, Saylorsburg, PA from June to August, 2003. The letter in parentheses indicates the month, June (JUN), July (JUL), August (AUG), that the bacteria were isolated in and the number indicates the number of times that the bacteria were isolated that month. Site 1 is located 100 feet from the reservoir, site 2 is 2 miles downstream from site 1 and site 3 is Saylor Lake fed by Ross Common Creek.

Site 1	Site 2	Site 3
Aeromonas hydrophila (JUL 1)	*Aeromonas hydrophila* (AUG 1)	*Aeromonas* sp. (AUG 1)
Alcaligenes sp. (JUN 1, AUG 2)	*Alcaligenes* sp. (AUG 2)	*Alcaligenes* sp. (AUG 1)
Citrobacter freundii (JUN 1)	*Citrobacter freundii* (JUN 1, JUL 1)	*Citrobacter freundii* (AUG 2)
Escherichia coli (JUN 1, JUL 1, AUG 2)	*Escherichia coli* (JUL 1, AUG 2)	*Escherichia coli* (JUN 1, JUL 1, AUG 2)
Klebsiella oxytoca (JUN 1, JUL 1, AUG 2)	*Klebsiella oxytoca* (JUL 1, AUG 1)	*Klebsiella oxytoca* (JUN 1, JUL 1, AUG 2)
Pantoea agglomerans (JUN 1)	*Klebsiella pneumoniae* (AUG 1)	*Pseudomonas* sp. (JUL 1)
Serratia marcescens (JUL 1)	*Pseudomonas aeruginosa* (JUL 1)	*Pseudomonas aeruginosa* (JUL 1, AUG 2)
	Serratia marcescens (JUL 1, AUG 2)	*Pseudomonas stutzeri* (JUN 1)
		Serratia sp. (JUN 1, JUL 1, AUG 2)

Table 2. Gram-negative bacteria isolated during June, July and August, 2003.

June	July	August
Alcaligenes sp.	*Aeromonas hydrophila*	*Aeromonas hydrophila*
Citrobacter freundii	*Citrobacter freundii*	*Alcaligenes*
Escherichia coli	*Escherichia coli*	*Citrobacter freundii*
Klebsiella oxytoca	*Klebsiella oxytoca*	*Escherichia coli*
Pantoea agglomerans	*Pseudomonas* sp.	*Klebsiella oxytoca*
Pseudomonas stutzeri	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Serratia sp.	*Serratia* sp.	*Klebsiella* sp.
	Serratia marcescens	*Serratia* sp.
		Serratia marcescens

Antibiotic resistance may not develop as a single event, but may involve a complex series of events (McArthur and Tuckfield 2000). The two types of resistance which occur in bacteria are intrinsic and extrinsic resistance. Ash et al. (2002) reported on antibiotic resistance of Gram-negative bacteria in rivers in the United States. The major genera isolated in that study were *Acinetobacter*, *Alcaligenes*, *Citrobacter*, *Pantoea*, and *Pseudomonas*. *Serratia*, *Klebsiella*, and *Proteus* were also isolated but less frequently than the other organisms. In our study *Alcaligenes*, *Citrobacter*, *Pantoea*, *Pseudomonas*, *Klebsiella*, and *Serratia*, were isolated. *Aeromonas* spp. are known to be intrinsically susceptible to all antibiotics active against non-fastidious Gram-negative bacilli, except for many beta-lactams. In this study, *Aeromonas hydrophila* was resistant to penicillin, ampicillin, oxacillin, tetracycline, and intermediate resistance occurred with methicillin and streptomycin. *Aeromonas hydrophila* was susceptible to oxytetracycline. Chloramphenicol resistance is an extremely rare trait in *Aeromonas* spp. (Montoya et al. 1992, Goni-Urriza et al. 2000), however, we report intermediate resistance in this genus isolated from Ross Common Creek. *Pantoea agglomerans*, is common in orchards associated with the outer surface of apples (Schnabel and Jones 1999), it is reported to be sensitive to aminoglycosides, chloramphenicol, tetracyclines, nalidixic aid, and nitrofurantoin. In this study *Pantoea agglomerans* and *Pseudomonas* sp. were resistant to chloramphenicol.

The majority of tetracycline (*tet*) resistant genes in bacteria have been associated with mobile plasmids, and transposons (Chopra and Roberts 2001). *Serratia* sp. was resistant to tetracycline in the majority of the isolates in this study, however, plasmids were not isolated from *Serratia* sp. According to Goni-Urriza et al. (2000) resistance determinants were considered to be chromosomally located in the absence of plasmids. It is possible that the tetracycline resistance exhibited by *Serratia* sp. in this study is chromosomally mediated.

Agricultural runoff is a major source of nutrients, pesticides and enteric microorganisms to surface and ground waters. Bacteria with intrinsic resistance to antibiotics are found in nature. Such organisms may acquire additional resistance genes from bacteria introduced into soil or water, and the resident bacteria may be the reservoir or source of widespread resistant organisms found in many environments. The results presented here have limitations and must be considered in light of the fact that many aquatic organisms are probably nonculturable. The bacteria that cannot be cultured may be part of the reservoir of resistance genes as well.

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Journal of the Pennsylvania Academy of Science 82(1): 7–11, 2008

THE INVASIVE NEW ZEALAND MUD SNAIL (*POTAMOPYRGUS ANTIPODARUM*) FOUND IN STREAMS OF THE LAKE ONTARIO WATERSHED[1]

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ABSTRACT

The invasive New Zealand mud snail (*Potamopyrgus antipodarum*) has been established in the Laurentian Great Lakes since at least 1991 and in the western United States since the late 1980s. In the western U.S. this snail has spread rapidly in rivers and streams where it dominates biomass in some locations. In Lake Ontario this species has been, until now, confined to water deeper than 4m. Here we report the first finding of the New Zealand mud snail in streams in the Great Lakes region. The snail was found in one of five sites examined in northwest New York. This finding demonstrates the continued spread of this species. The presence of the snail in lotic waters in the Great Lakes region may lead to substantial ecosystem changes similar to those observed in the western U.S. and elsewhere. It is advised that streams emptying into the Great Lakes be monitored for the presence of this species. [J PA Acad Sci 82(1): 7–11, 2008]

INTRODUCTION

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray 1843), is a global invasive species with well-established populations in the British Isles (Wallace 1985), mainland Europe (e.g., Stadler et al. 2005), Australia (Ponder 1988), Japan (e.g., Shimada and Urabe 2003), and North America (Bowler 1991; Zaranko et al. 1997). In North America there are two main populations, one centered in the rivers and streams of the western United States (Bowler 1991; Richards et al. 2001; Kerans et al. 2005), and one centered in the Laurentian Great Lakes (Zaranko et al. 1997; Grigorovich et al. 2003; Levri et al. 2007). Invasive populations, including those in North America, appear to be clonal, the result of parthenogenetic reproduction (Proctor et al. 2007). The snail in New Zealand exists in mixed populations of clonal and sexual individuals (Lively 1987). There are at least three different clones established in North America.

Two different clones have been discovered in the western U.S. The dominant western clone appears to be identical to a clone found in Australia (Emblidge Fromme and Dybdahl 2006; Proctor et al. 2007), and it was probably introduced by stocking fish (Bowler 1991; Bowler and Frest, 1992). In the western U.S. the snail is a nuisance species in rivers and streams. It was first found in the late 1980s in the Snake River in Idaho (Bowler 1991) and has since spread to every western U.S. state west of the Rocky Mountains except for New Mexico (Proctor et al. 2007). Densities of the snail in some locations have been found to be 500,000 per m^2 (Hall et al. 2003).

Based on molecular data (Emblidge-Fromme and Dybdahl 2006) from one site (Wilson, NY–Lake Ontario), it appears that one genotype exists in the Great Lakes. This clone is identical to one of the two clones found in mainland Europe (Emblidge-Fromme and Dybdahl 2006; Proctor et al. 2007) where it is found in a wide range of freshwater habitats including lakes and streams (Dybdahl pers. comm.; Jokela pers. comm.). It is likely that it was introduced via international shipping and may have been the result of ballast water introduction. In the Great Lakes, the snail was first discovered in 1991 in the northeast and southwest portions of Lake Ontario and in parts of the St. Lawrence River (Zaranko et al. 1997). The geographic range of the snail has since expanded within Lake Ontario (Levri et al. in press) and now includes Lake Superior (Grigorovich et al. 2003), and Lake Erie (Levri et al. 2007). Population densities of the snail in the Great Lakes vary substantially within and between years with densities ranging from <10 per m^2 to about 5000 per m^2 (Zaranko et al. 1997; Levri et al. in press). The distribution within the lakes ranges from 4 m to at least 40 m in depth (Zaranko et al. 1997; Levri et al. in press). The fact that the snail has not been found in shallow water (< 4 m) may be a reason why, until now, they have not been found in rivers or streams emptying into the Great Lakes.

Relatively little is known about the ecological impacts of the snail. What is known has been discovered by studying populations in streams and rivers in the western U.S. and in Australia. In streams and rivers, *P. antipodarum* has been shown to compete with native invertebrates, such as mayflies (Cada 2004), possibly by inhibiting colonization (Kerans et al. 2005) and/or consuming a substantial proportion of primary production (Hall et al. 2003; Cada 2004; Hall et al. 2006). The snail dominates secondary productivity in several locations (Hall et al. 2006) and negatively impacts higher trophic levels (Cada 2004; Hall et al. 2006). Trout have been shown to feed upon *Potamopyrgus* (Vinson et al. 2007), and the diets of trout and sculpin have been shown to change in

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response to increased densities of the mud snail in nature (Cada 2004). This effect was probably due to the change in community composition associated with the snail. Consumption of the snails by fish in streams may decrease growth rates and result in weight loss because the snail is a poor food source compared to native prey (Vinson et al. 2007). The snails have also been found to alter the carbon and nitrogen cycling in lotic habitats (Hall et al. 2003). Specific effects in the Great Lakes have yet to be determined. One reason for the lack of knowledge about the effects of the snails in the Great Lakes is due to the relatively simultaneous introduction of zebra mussels to the region, which makes attributing ecological changes to *Potamopyrgus* difficult. One of the primary concerns about the Great Lakes population is the potential for the species to spread from the lakes into lotic waters where substantial ecological effects have been noted (Hall et al. 2003; Cada 2004; Kerans et al. 2005).

Here we report the first occurrence of *Potamopyrgus antipodarum* in shallow lotic waters in the Great Lakes region.

METHODS

Five streams emptying into Lake Ontario in Niagara County, NY were sampled on 5 June 2007 along roadways. Rocks and other debris ranging in size from 8 cm^3 to 1000 cm^3 were picked up out of riffle and pool habitats and examined for the presence of *P. antipodarum*. If no mud snails were discovered after twenty minutes at a location the search was concluded. The presence or absence of New Zealand mud snails was noted and a sample was collected where they were found; however, no density estimates were made. Snails collected were identified using *The Freshwater Snails (Mollusca: Gastropoda) of New York State* by Jokinen (1992) and compared to a personal reference collection of *P. antipodarum*. All sites examined were characterized by relatively shallow water (<1 m) and easy access to the streams from the road. Locations of each site are listed in Table 1.

RESULTS

P. antipodarum (Figure 1) was found in one of the five sites examined (Figure 2; Table1). At site 1, they were easily found and collected from large rocks by the tunnel on the north side of the road (Rt. 18F). Approximately 75 snails were collected from all sides of five large rocks examined. Although populations in the Great Lakes exhibit smooth and keeled morphs, no shell armature was found on the snails collected in the streams. The majority of the snails from site 1 possessed a dark encrusting material on the shells, however (Figure 1).

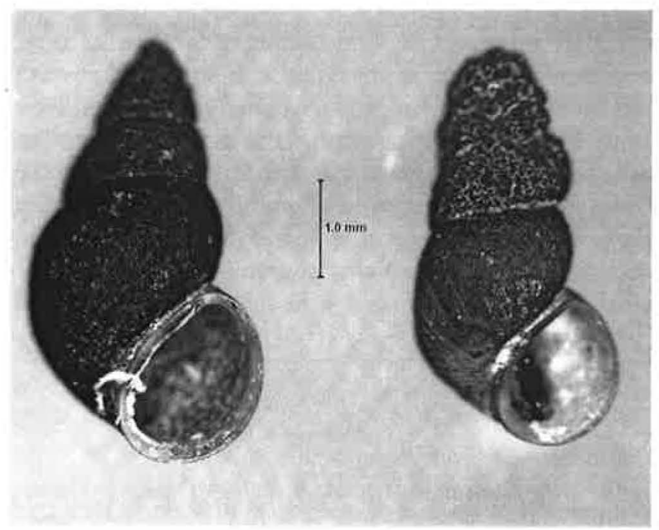

Figure 1. Two individual *P. antipodarum* snails collected from site 1. The snail on the right is encrusted with a hard granular material.

Table 1. Locations of each sampling site in streams emptying into Lake Ontario in Niagara County in western New York.

Site #	Stream	Latitude	Longitude	*Potamopyrgus* found?	Site distance (by way of stream) from Lake Ontario (km)	Stream width; depth sampled	Substrate	Watershed characteristics
1	Unnamed stream near Youngstown, NY	43° 16.215'	79° 01.256'	Yes	0.5	2.5 m; <0.5 m	Large Cobble	Mixed agricultural and forest
2	Fourmile creek	43° 16.089'	79° 00.229'	No	1.4	7 m; <0.5 m	Cobble and silt	Mixed agricultural and residential
3	Twelvemile creek	43° 16.725'	78° 53.406'	No	7.6	~5 m; <0.5 m	Mostly silt, some cobble	Mostly agricultural
4	East Branch	43° 18.434'	78° 49.880'	No	2.4	~6 m; <0.5 m	Cobble	Mostly agricultural
5	East Branch of Eighteen mile creek	43° 13.150'	78° 41.734'	No	16	9 m; <0.5 m	Cobble	Mixed agricultural and forest

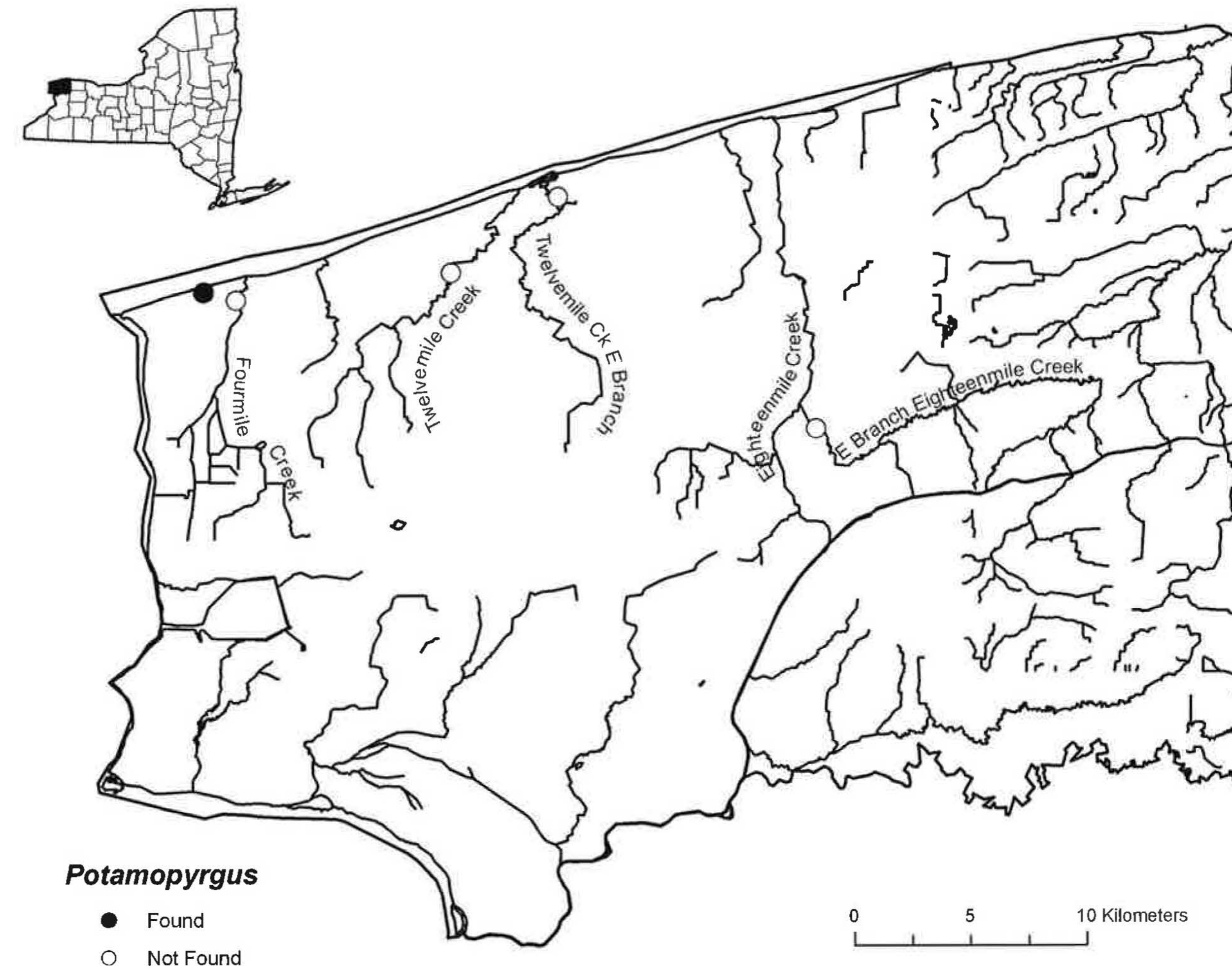

Figure 2. Map Niagara County, NY indicating the sites surveyed for *P. antipodarum*.

DISCUSSION

To our knowledge, this is the first finding of the New Zealand mud snail in streams in the eastern United States. The snail has not been found in other large collections from streams in this region based upon word from several other researchers and organizations (Jackson pers. comm.; Suprenant pers. comm.; Strayer pers. comm.). This species can be rather inconspicuous at low densities, however. Thus we suggest that researchers re-examine archived collections from the Lake Ontario watershed.

The genotype of *Potamopyrgus antipodarum* from this stream location was found to be identical to snails from Lake Ontario (Dybdahl unpublished data) indicating that the snail in the Great Lakes has invaded lotic habitats. In a limited survey of 14 streams emptying into Lake Ontario in upstate New York in 2003, *Potamopyrgus* was not found (Levri unpublished data). However, the sites sampled in the present study were not included in the 2003 survey. Site 1 is approximately 0.5 stream kilometers from Lake Ontario. It should be noted here that the sampling that revealed the presence of *Potamopyrgus* in streams was very limited. Thus it is likely that the snail is established in other streams and rivers in the region. More detailed surveys are planned.

Potamopyrgus antipodarum is a successful invader largely due to its reproductive capabilities and broad environmental tolerances (Proctor et al. 2007). Since invasive populations of this species reproduce parthenogenetically, only one snail is required to establish a new population. Snails can produce broods as large as 70 individuals (Levri unpublished data). Females can produce offspring during the entire growing season (Schreiber et al. 1998), and reproductive maturity can be reached in as little as 3 months (Jokela pers. comm.). In New Zealand and in invaded areas, the snail is found in a wide variety of aquatic environments, including rivers, streams, lakes, and springs, and can be found in a wide variety of habitats within each type of environment including fine and course substrates (Cunha and Moreira 1995; Kerans et al. 2005), vegetation (Dorgelo 1987; Quinn et al. 1996), littoral shorelines (Quinn et al. 1996), and deep benthos (Zaranko et al. 1997; Levri et al. 2007). *Potamopyrgus* can withstand a wide range of temperatures ranging from 0°C (Hylleberg and Siegismund 1987) to 32°C (Quinn et al. 1994). Cold winter temperatures have been observed to result in high mortality (Zaranko et al. 1997). The snail also tolerates a wide range of salinities, as one population in the Columbia River estuary exists in an environment that fluctuates between completely fresh (0 ppt) to seawater (32 ppt) (Dybdahl and Kane 2005). It can also tolerate periodic scouring flows in lotic systems (Holomuzki and Biggs 2000).

In New Zealand, densities in streams are highly dependent on flood frequency with decreasing densities with increasing flood frequency, and densities rarely exceed 1000 per m^2 (Holomuzki and Biggs 1999). The densities of invasive populations in streams show substantially more variation. Densities of 30,000 per m^2 in the western U.S. are common (Richards et al. 2001; Dybdahl pers. comm.) and some locations have densities an order of magnitude larger (Kerans et al. 2005). In Australia, *Potamopyrgus* densities are positively correlated with human disturbance (Schreiber et al. 2003). Some studies have found that moderately eutrophic waters favor *Potamopyrgus* productivity (Dorgelo 1987, Scott et al. 1994). In one experiment, *P. antipodarum* was shown to prefer gravel compared to sand, fine sediment, and pebbles, but some snails were found on all substrates tested (Lysne and Koetsier 2006). In lotic systems, it appears that densities are highest in streams with relatively constant flow and temperatures and relatively high primary productivity (Hall et al. 2003; Richards 2004; Kerans et al. 2005).

Now that the snail is established in streams, dispersal can occur both naturally and by hitchhiking on recreational stream users. This type of dispersal is likely the primary method of dispersal in the western U.S. (Proctor et al. 2007). The snail can easily be caught in fishing equipment, clothing, footwear, etc. It also is capable of surviving periods of at least 24 hours of complete drying (Levri unpublished data) and, if kept moist, can be viable for 50 days or more (Winterbourn 1970). To reduce the possibility of spreading, it has been suggested that water users:

1. clean all mud and debris from clothing and gear after using a water body containing the snail.
2. put gear in a freezer for 6–8 hours, hot water (>120°C) for a few minutes, or in a dry environment of at least 84°C for at least 24 hours before using gear in a new water body (Richards et al. 2004; Proctor et al. 2007).

Since some of the above methods may not always be practical, gear and footwear can be treated with some common household cleaning solutions such as Formula 409®, Pinesol®, and ammonia (see Proctor et al. 2007 for more details).

Because of the rapid spread of the snail in the western U.S. and the snail's ability to tolerate a wide range of habitats, we expect that a similarly rapid increase in range will occur in the eastern U.S. and southern Canada. Using GARP models, Loo et al. (2007) predicted that *Potamopyrgus* has a potential range of most of the continental United States, southern Canada, and much of inland Mexico.

We suggest that efforts begin immediately in the Great Lakes region and across eastern North America to educate recreational users, scientists, educators, and other water users about the potential harm that this species could cause and about measures to reduce their spread. Aside from streams emptying into Lake Ontario, it is advised that streams be monitored for the presence of this species in watersheds of Lake Erie and Superior. It is likely that this species will spread rapidly in rivers and streams of the Northeast United States and Canada.

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INVENTORY OF MAMMALS AT VALLEY FORGE NATIONAL HISTORICAL PARK[4]

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ABSTRACT

We conducted an inventory of mammals at Valley Forge National Historical Park (VAFO), Pennsylvania, from March–October 2004. Our objectives were to update existing records of mammalian species observed previously at the park and add records of species not observed previously. Live-trapping, spotlight surveys, and opportunistic observations were used to inventory mammals; we did not employ special trapping efforts for bats, as a separate inventory project was planned for that taxonomic group. We documented 1,098 encounters of 20 species of mammals: Virginia opossum (scientific names are in the Appendix), masked shrew, northern short-tailed shrew, big brown bat, eastern cottontail, eastern chipmunk, gray squirrel, red squirrel, southern flying squirrel, woodchuck, white-footed mouse, meadow vole, Norway rat, meadow jumping mouse, common raccoon, coyote, red fox, feral cat, striped skunk, an unidentified weasel, and white-tailed deer. Big brown bat, coyote, and feral cat represent new records at VAFO; the weasel (ermine or long-tailed weasel) would be a new record, but the sighting was not reliable and is considered an unconfirmed sighting. We did not encounter any mammalian species federally listed as threatened or endangered or state listed as vertebrates of special concern in Pennsylvania. We recommend that future mammalian-inventory efforts at VAFO be based on a long-term monitoring program that involves the survey methods we used plus additional methods that are well-suited for detecting species that we did not encounter (e.g., eastern, hairy-tailed, and star-nosed moles; least and smoky shrews; muskrat; mink). Lastly, we recommend active management of feral cats at VAFO to benefite native wildlife.

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INTRODUCTION

The National Park Services (NPS) has determined that park managers need comprehensive information about biological resources to maintain biodiversity and natural ecosystems in parks (NPS 2000). As national parks become more insular from increased habitat fragmentation, they will be increasingly valuable for the long-term maintenance of faunal diversity and the functional integrity of landscapes and ecosystems in the eastern United States (Ambrose and Bratton 1990, Yahner 2000). One of the first steps required to achieve the NPS goal of conserving biodiversity in national parks is to conduct baseline inventories in each park to determine which species are present.

Prior to 2004, only several biological research projects involving mammals were conducted at Valley Forge National Historical Park (VAFO), Pennsylvania. One study (Cypher et al. 1985) was an inventory of flora and fauna in only a portion of the park. Another (Yahner et al. 1997) investigated the effectiveness of trapping protocols for small mammals. A third study (Lovallo and Tzilkowski 2003) examined abundance and movement of white-tailed deer (scientific names are provided in the Appendix). Additional data about presence of mammals at VAFO is limited to unverified sightings reported by park staff and the general public. Although those studies and reports contribute some knowledge about the presence and distribution of mammalian species at VAFO, none represents a baseline, park-wide inventory of those species.

As part of a broader study examining biological resources at VAFO (Yahner et al. 2006a, 2006b), we conducted a park-wide inventory of mammals to (1) reconfirm presence of species reported previously at the park, and (2) document presence of species that were not reported previously, or have become established at the park only recently. Our goal was to update and increase current knowledge about presence of mammalian species at VAFO, thereby providing baseline data that may be considered during the development of a long-term monitoring program at the park.

MATERIALS AND METHODS

Study Area

VAFO consists of 1,408 ha in Montgomery and Chester counties, southeastern Pennsylvania; approximately 1,316 ha are federally owned. The park is surrounded by industrial, commercial, and residential development, and major highways are to the north, south, and east. Topography is rolling uplands and low hills; elevation ranges from 18–161 m (Tiebout 2003). The majority of acreage under federal ownership is relatively mature forest, and grassland, although young woodlots and maintained areas (lawn, development, etc.) are common (Table 1). The Schuylkill River is the major drainage and traverses the park from west to east.

Our park-wide inventory of mammals was conducted concurrently with two site-specific inventories (Yahner 2006a, 2006b) of birds, herpetofauna, woody plants, and mammals. Although those two inventories had objectives particular to each site, we intended to use mammal data collected during those inventories to help achieve objectives of the park-wide mammal inventory. Therefore, we subdivided VAFO into four sectors for collection of data: 1) the Asbestos Release Site (ARS), 2) reference plots associated with the ARS, 3) the Schwoebel tract, and 4) other areas within VAFO. The first sector comprised 15 areas (39 ha combined) contaminated with subsurface asbestos (Foster Wheeler Environmental Corporation 2003) and inventoried specifically for herpetofauna and small mammals (Yahner et al. 2006a). The second sector comprised five reference plots (approximately 4 ha combined) located outside the ARS (but within VAFO) and also inventoried for herpetofauna and small mammals (for comparison to the ARS; Yahner et al. 2006a). The third sector, the Schwoebel tract, was a 29-ha inholding of VAFO that was officially acquired by the NPS in 2004 and inventoried specifically for birds, herpetofauna, woody plants, and mammals (Yahner et al. 2006b). The fourth sector comprised all other areas of VAFO and was inventoried specifically for mammals. Cover types (Table 1) in the ARS and reference plots were predominantly successional and grassland. The Schwoebel tract consisted mainly of the old nursery type. Major cover types in other areas of VAFO were forest, grassland, and anthropogenic types (e.g., lawn, development, and cropland).

Sampling Design

We developed a list of 46 mammalian species for which presence at VAFO may be expected (see Appendix). We achieved this list of "expected species" by reviewing the NPSpecies database of the NPS (NPS 2003), which documents occurrence of species in national parks, and by inspecting data from various sources (e.g., published reports and range maps [Burt and Grossenheider 1980, Merritt 1987]). Our sampling design was then developed to maximize our ability to document presence of as many of those species as possible while considering logistical and budgetary constraints.

We focused our sampling efforts in cover types that we presumed to be most suitable for species on our expected list. Therefore, most sampling occurred in the forest, successional, old nursery, grassland, and wet meadow types. However, we also sampled the developed type for species that occur in or near human developments (e.g., house mouse, Norway rat, southern flying squirrels, bats).

Sampling occurred for various lengths between March October 2004 and consisted of live-trapping, roadside spotlight surveys, and opportunistic observations of animals or their signs (e.g., tracks, fur, burrows, and scats). We selected this combination of protocols because we believed it would be the most efficient way to maximize the acreage and number of cover types sampled. In addition, these protocols are less labor-intensive and less likely to result in animal mortality relative to other methods (e.g., pitfall- and snap-trapping). Opportunistic observations were the only means by which we attempted to inventory bats, as identification of bats often requires specialized training and an intensive sampling effort. Furthermore, a separate study (Hart 2006) was scheduled for inventory of bats at the park.

Live-trapping was conducted with use of three trap types: small (17 × 6 × 5 cm [6.5 × 2.5 × 2.0 in]) Sherman, medium (41 × 13 × 13 cm [16 × 5 × 5 in]) Tomahawk, and large (81 × 26 × 32 cm [32 × 10 × 12 in]) Tomahawk. Sherman traps were baited with peanut butter, and Tomahawk traps were baited with ground beef, tuna, or a mixture of peanut butter

Table 1. Cover types at Valley Forge National Historical Park, Pennsylvania (modified from Lundgren et al. 2002; see Yahner et al. 2006c).

Cover Type	Description	Area (ha)
Forest	Relatively mature, closed-canopy woodlot dominated by true overstory species (e.g., white pine [*Pinus strobus*], red oak [*Quercus rubra*]); relatively sparse understory and herbaceous cover	374
Grassland	Grassy area mowed not more than once per year	362
Successional	Old field or relatively young woodlot with open or patchy canopy; relatively dense understory and/or herbaceous cover	181
Lawn	Grassy area mowed frequently (weekly or bi-weekly)	135
Developed	Building, road, or parking lot	120
Water	River, stream, or pond	53
Cropland	Agricultural field consisting of row crops (e.g., corn)	45
Old Nursery	Abandoned tree nursery; relatively early successional community consisting primarily of non-native, cultivated vegetation	33
Ornamental Grove	Planted shade trees in areas maintained by frequent mowing	9
Wet Meadow	Seassonally flooded grassland	5
Talus Slope	Rocky outcrop on hillside; vegetation absent	<1

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Table 2. Sampling effort by cover type, trap type, and desired species for live-trapping of mammals at Valley Forge National Historical Park, PA, 2004.

Cover Type	Trap Type[a]	No. Trap Nights[b]	Desired Species/Groups[c]
Forest	S	293	smoky shrew, small mammals, nuisance
	MT	179	ermine, long-tailed weasel, e. fox squirrel, s. flying squirrel, nuisance
	LT	18	mink
Successional	S	411	small mammals, nuisance
	MT	5	ermine, long-tailed weasel, e. fox squirrel
	LT	5	nuisance
Old Nursery	S	188	small mammals, nuisance
	MT	10	ermine, long-tailed weasel, e. fox squirrel
	LT	10	nuisance
Grassland	S	404	s. bog lemming, small mammals
Wet Meadow	S	195	s. bog lemming
Developed	S	30	house mouse, small mammals, nuisance
	MT	33	s. flying squirrel
	LT	14	feral cat, nuisance

[a]S = Sherman (17 × 6 × 5 × cm [6.5 × 2.5 × 2.0 in]); MT = medium Tomahawk (41 × 13 × 13 cm [16 × 5 × 5 in]); LT = large Tomahawk (81 × 26 × 32 cm [32 × 10 × 12 in]).
[b]Trap night = one trap set over one night.
[c]Small mammals = all species the size of eastern chipmunk or smaller; nuisance = common raccoon, eastern chipmunk, white-footed mouse, and other species suspected of disturbing traps (or their baits) that were set for capture of particular target species (i.e., some traps were set intentionally for nuisance species in an effort to reduce problems with trap disturbance).

and oats. Sherman traps were used to capture mammals the size of eastern chipmunk or smaller, medium Tomahawk traps were used to capture mammals between the size of eastern chipmunk and striped skunk, and large traps were used to capture mammals the size of rabbits or larger. Total number of trap nights (trap night = one trap set over one night) was 1,521,227 and 47 for Sherman, medium Tomahawk, and large Tomahawk traps, respectively (Table 2).

Trapping consisted of two strategies. The first strategy consisted of using the small Sherman traps to sample exclusively for small mammals at the ARS, reference plots, and the Schwoebel tract in conjunction with the two site-specific inventory projects (Yahner et al. 2006a, 2006b). Sampling in those three sectors occurred by placing traps at 10-m intervals along transects of varying number and length, based on an attempt to distribute trapping effort proportionally to area (ha) of each cover type within a given sector (see Yahner et al. 2006a, 2006b for transect configurations). Trapping occurred during the course of 1 week in both July and October, with traps on a given transect being set for one night per month. Total trap nights (trap nigh = one trap set over one night) under this strategy was 578 at the ARS, 200 at reference plots, and 230 at the Schwoebel tract.

The second strategy consisted of using all three trap types at locations elsewhere in the park. Trapping was conducted approximately one week per month during June-October. Trapping effort was concentrated mainly in forest, wet meadow, and developed cover types because they were not well-represented in the other three sectors of the study area. We developed a list of "target species" to capture, which consisted of eastern fox squirrel, feral cat, house mouse, mink, smoky shrew, southern bog lemming, southern flying squirrel, and weasels (ermine, long-tailed weasel). Although feral cat was not an expected species, we designated it a target species mid-way through the study because cats were observed in the southeast sector of the park but were unable to be identified as domestic or feral without closer inspection (e.g., for presence of a collar). The target list excluded (1) species that we had already documented using other methods, (2) species that we expected to capture using the first sampling strategy (e.g., grassland and early-successional woodland species smaller than eastern chipmunk), (3) species that are generally very difficult to live-trap (e.g., American beaver, bats, muskrat, gray fox), and (4) species for which suitable habitat seemed to be absent at the park (e.g., Appalachian cottontail).

Twenty-nine sampling points were established at locations where target species were most likely to occur in the park. These sections were based on habitat requirements of desired species. We sampled for house mouse at a building occupied by humans (n = 6 trap nights), for feral cat at an abandoned barn (n = 3 trap nights), for southern bog lemming in a grassland and several wet meadows (n = 210 trap nights), for mink along the shoreline of a creek (n = 18 trap nights), for smoky shrew in mature forest (n = 120 trap nights), and for ermine/long-tailed weasel (n = 112 trap nights), southern flying squirrel (n = 111 trap nights), and eastern fox squirrel (n = 190) in several cover types. Several traps set for the two squirrel species were placed 1–4 m off the ground (in trees and on the roof of a building).

At each sampling point for target species, 1–30 traps were set for a period of 1–6 consecutive days. When we used more than one trap type at a sampling point, we spaced individual traps 1–5 m apart. However, when we used only Sherman traps at a sampling point, we placed traps at 10-m intervals along one or two transects centered on the sampling point. Number and type of traps, number of trapping periods, and length (days) of trapping period at each sampling point were chosen arbitrarily, based mainly on logistical compatibility with concurrent inventory efforts (Yahner, et al. 2006a, 2006b), and because effectiveness of trapping effort at a given point was difficult to evaluate (e.g., because of difficulty in trapping particular target species, uncertain likelihood that target species would occur at a sampling point, disturbance of traps by non-target species, and variable weather conditions). Disturbance of traps and baits by non-target species (e.g., common raccoon, eastern chipmunk, and white-footed mouse) became such a problem that we set additional traps for those species, presuming that such capture and sequestration would increase the probability of other traps at the sampling point remaining open and baited for the target species.

For both trapping strategies, traps were checked daily, and animals captured were identified to species and released at the point of capture (except for feral cats, which were left in the care of park personnel). Traps were removed from the field between trapping sessions.

We used roadside spotlight surveys to detect species that are either difficult to live-trap (e.g., foxes) or are too large to be trapped in the types of traps we used (e.g., white-tailed deer). Nine spotlight surveys traversing a total of 117 km were conducted March–October by driving slowly (≤40 km/h) along park roads after dusk (between 1750 and 0035 hrs) and using headlights and a spotlight to view mammals in or near the roadway. The survey route at the Schwoebel tract (n = 3 surveys) consisted of a 0.3-km driveway through the interior of the tract. Survey routes elsewhere at VAFO (n = 6 surveys) were variable and ranged from 10.5–26.7 km. For each animal encountered, we recorded the species and number of individuals observed.

Opportunistic observations of mammal species were noted throughout the study (March–October) to document species that had not been observed during live-trapping and spotlight surveys, and to provide additional data that park managers were interested in acquiring. We recorded an opportunistic observation of a species whenever (1) the species had not yet been documented during the inventory, (2) the species was considered to be relatively uncommon or rare at VAFO, (3) the individual was unique in some fashion (e.g., deformed, marked, etc.), or (4) a large group (arbitrarily, >10 individuals) was encountered. Generally, we were present at the park and noting such observations approximately two weeks per month.

RESULTS

We documented 1,098 encounters of 20 mammalian species at VAFO (Table 3), plus an encounter of an unidentified weasel (ermin or long-tailed weasel). Records comprised five encounters of opossum, 34 encounters of shrews (n = 2 species), one encounter of a bat (n = 1 species), five encounters of cottontails (n = 1 species), 355 encounters of rodents (n = 9 species), and 635 encounters of white-tailed deer. Based on our review of the NPSpecies database (NPS 2004), three species represent new records at VAFO: big brown bat, coyote, and feral cat. Other bats were encountered during the study, but the observations consisted of flying individuals, and identification to species was not achieved. The big brown bat observation consisted of an individual that was captured by hand as it roosted on the side of a building.

We encountered more individuals (n = 405) in lawn cover than in any other cover type, but those observations comprised only three species (common raccoon, red fox, and white-tailed deer). Most other encounters occurred in grassland (314 individuals, 9 species), successional (181 individuals, 14 species), old nursery (61 individuals, 11 species), forest (64 individuals, 8 species), developed (40 individuals, 11 species), and wet meadow cover (29 individuals, 1 species) (species-by-species accounts are available in Yahner et al. 2006c).

We encountered more individuals via spotlight surveys (n = 494) than by any other protocol. Spotlight surveys yielded observations of only four species (woodchuck, common raccoon, red fox, and white-tailed deer) and did not account for species not detected by other means. In contrast, opportunistic observations yielded 284 individuals of 19 species, including nine species (masked shrew, big brown bat, eastern cottontail, meadow jumping mouse, Norway rat, red squirrel, coyote, and striped skunk) not encountered by other protocols. Trapping yielded 320 individual encounters of nine species, including one species (southern flying squirrel) that was not encountered by other protocols.

DISCUSSION

We documented presence of three species (big brown bat, coyote, and feral cat) for which previously confirmed records at VAFO did not exist (NPS 2004). We also updated confirmed records in the NPSpecies database (NPS 2004) for three species (Norway rat, red squirrel, and southern flying squirrel), which were last documented by McKeever (1979), four species (Virginia opossum, northern short-tailed shrew, masked shrew, and meadow jumping mouse) documented by Cypher et al. (1985), nine species (eastern cottontail, eastern chipmunk, gray squirrel, meadow vole, white-footed mouse, woodchuck, common raccoon, red fox, and striped skunk) documented by Yahner et al. (1977), and one species (white-tailed deer) documented by Lovallo and Tzilkowski (2003).

In addition to producing updated mammalian records, our inventory increased knowledge about presence of particular species at VAFO, sectors and cover types of the park where those species can be found, and sampling protocols that may be effective for future monitoring of the species. However, we caution that our results are limited in their usefulness for estimating other population parameters (e.g., relative abun-

Table 3. Number of individual encounters[a] of each mammal species, by sector, recorded during the inventory of mammals at Valley Forge National Historical Park, Pennsylvania, 2004.

Common Name	ARS[b]	REF[c]	SCHW[d]	VAFO[e]	Total
Didelphids					
Virginia opossum			2	3	5
Insectivores					
masked shrew		12	4		16
northern short-tailed shrew	12	5	1		18
Bats					
big brown bat				1	1
Lagomorphs					
eastern cottontail	1	1	3		5
Rodents					
eastern chipmunk		1		7	8
gray squirrel	15	3	6	1	25
meadow jumping mouse			1		1
meadow vole	30	26	10	31	97
Norway rat				1	1
red squirrel	2	1			3
southern flying squirrel				1	1
white-footed mouse	88	28	44	49	209
woodchuck	6	1	2	1	10
Carnivores					
common raccoon	4	4	2	14	24
coyote	1				1
feral cat			1	6	7
red fox	3	2	10	15	30
striped skunk				1	1
Artiodactyls					
white-tailed deer	148	13	5	469	635
Total no. encounters	310	97	91	600	1,098
Total no. species	11	12	13	14	20

[a]Encounter = individual animal or its sign observed at a particular time; animals were not marked, so totals may include multiple observations of some individuals. Excludes encounters not identified to species.
[b]Asbestos Release Site, from the Asbestos Release Site inventory project (Yahner et al. 2006a).
[c]Reference plots, from the Asbestos Release Site inventory project (Yahner et al. 2006a).
[d]Schwoebel tract, from the Schwoebel inventory project (Yahner et al. 2006b).
[e]Other locations within Valley Forge National Historical Park.

dance) because our sampling design consisted mainly of opportunistic observations and non-standardized methods of spotlighting and trapping. Factors that limit application of our data include possible double-counting of individuals, differences in detection probability among species, and no assurance that proportions of populations detected over time and space were consistent (Conroy 1996). Species not encountered included five moles and shrews (eastern mole, hairy-tailed mole, least shrew, smoky shrew, and star-nosed mole), nine bats (eastern pipistrelle, evening bat, hoary bat, northern myotis, little brown myotis, red bat, Seminole bat, silver-haired bat, and small-footed myotis), one cottontail (Appalachian cottontail), eight rodents (American beaver, common muskrat, deer mouse, eastern fox squirrel, house mouse, southern bog lemming, southern red-backed vole, and woodland vole), and four carnivores (ermine, long-tailed weasel, mink, and gray fox). There are historical records of confirmed sightings of only four of those species (star-nosed mole, common muskrat, deer mouse, and house mouse) prior to our inventory (NPS 2004).

Conservation Implications

We did not encounter any species federally listed as threatened or endangered (USFWS 2005) or state listed as vertebrates of special concern in Pennsylvania (PANHP 2006). Therefore, management activities at VAFO would not be expected to impact any mammalian species of conservation concern. However, managers should keep in mind that VAFO does overlap with the geographic ranges of six species (least shrew, evening bat, silver-haired bat, small-footed myotis, Appalachian cottontail, and eastern fox squirrel) that are of special concern in Pennsylvania (Burt and Grossenheider 1980, Merritt 1987, PANHP 2006).

Although our results are inconclusive, we do not recommend that the NPS invest additional resources into investigating possible presence of Appalachian cottontail, American beaver, eastern fox squirrel, deer mouse, or gray fox at VAFO unless there is a desire to manage those species specifically. Lack of suitable habitat at the park, combined with failure of our inventory to detect presence of the species, suggests that viable populations of those species are absent. Future efforts to document presence of least shrew, smoky shrew, southern bog lemming, southern red-backed vole, woodland vole, common muskrat, house mouse, ermine, long-tailed weasel, and mink might be served best by a long-term monitoring program, as future discovery of one or more of these species may be more a product of chance (e.g., seeing an animal run across a road) than of intensive sampling effort. Special surveys that utilize methodologies not included in our study (e.g., pitfall-trapping for shrews, and snare or foothold traps for mink and muskrat) could potentially yield observations of those species. However, we caution against destructive sampling when species that, if present, likely occur in low abundance (e.g., mink) are involved. Intensive pitfall-trapping may be worthwhile for updating the status of eastern, hairy-tailed, and star-nosed moles. If there is a need to further investigate the status of house mouse or Norway rat, we recommend trapping (live-traps or snap-traps, depending on management objectives) in buildings and other anthropogenic structures throughout the park.

Finally, The Wildlife Society encourages and supports the humane elimination of feral cat colonies because feral and free-ranging domestic cats are exotic predators that contribute significantly to the mortality of small mammals, birds, reptiles, and amphibians (TWS 2005). Impacts of feral and free-ranging domestic cats on native wildlife are exacerbated when such cats are fed by humans. Our inventory confirmed that the problem of feral cats inhabiting the park should be considered by park managers when designing and implementing management strategies for promotion and maintenance of natural diversity of wildlife and wildlife habitat.

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Appendix. Common and scientific names of expected (E) and observed (O) species of mammals at Valley Forge National Historical Park, 2004. Expected species were obtained by reviewing Burt and Grossenheider (1980), Merritt (1987), and NPS (2003).

Common Name	Scientific Name	Expected/Observed
American beaver	*Castor canadensis*	E
Appalachian cottontail	*Sylvilagus obscurus*	E
big brown bat	*Eptesicus fuscus*	E/O
common muskrat	*Ondatra zibethicus*	E
common raccoon	*Procyon lotor*	E/O
coyote	*Canis latrans*	E/O
deer mouse	*Peromyscus maniculatus*	E
eastern chipmunk	*Tamias striatus*	E/O
eastern cottontail	*Sylvilagus floridanus*	E/O
eastern fox squirrel	*Sciurus niger vulpinus*	E
eastern mole	*Scalopus aquaticus*	E
eastern pipistrelle	*Pipistrellus subflavus*	E
ermine	*Mustela erminea*	E
evening bat	*Nycticeius humeralis*	E
feral cat	*Felis catus*	O
gray fox	*Urocyon cinereoargenteus*	E
gray squirrel	*Sciurus carolinensis*	E/O
hairy-tailed mole	*Parascalops breweri*	E
hoary bat	*Lasiurus cinereus*	E
house mouse	*Mus musculus*	E
least shrew	*Cryptotis parva*	E
little brown myotis	*Myotis lucifugus*	E
long-tailed weasel	*Mustela frenata*	E
masked shrew	*Sorex cinereus*	E/O
meadow jumping mouse	*Zapus hudsonius*	E/O
meadow vole	*Microtus pennsylvanicus*	E/O
mink	*Mustela vison*	E
northern myotis	*Myotis septentrionalis*	E
northern short-tailed shrew	*Blarina brevicauda*	E/O
Norway rat	*Rattus norvegicus*	E/O
red bat	*Lasiurus borealis*	E
red fox	*Vulpes vulpes*	E/O
red squirrel	*Tamiasciurus hudsonicus*	E/O
Seminole bat	*Lasiurus seminolus*	E
silver-haired bat	*Lasionycteris noctivagans*	E
small-footed myotis	*Myotis leibii*	E
smoky shrew	*Sorex fumeus*	E
southern bog lemming	*Synaptomys cooperi*	E
southern flying squirrel	*Glaucomys volans*	E/O
southern red-backed vole	*Clethrionomys gapperi*	E
star-nosed mole	*Condylura cristata*	E
striped skunk	*Mephitis mephitis*	E/O
Virginia opossum	*Didelphis virginiana*	E/O
white-footed mouse	*Peromyscus leucopus noveboracensis*	E/O
white-tailed deer	*Odocoileus virginianus*	E/O
woodchuck	*Marmota monax*	E/O
woodland vole	*Microtus pinetorum*	E

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GEOLOGY OF THE GOAT HILL SERPENTINE BARRENS, BALTIMORE MAFIC COMPLEX, PENNSYLVANIA[1]

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ABSTRACT

The Goat Hill Serpentine Barrens tract is located in the southwestern corner of Chester County, Pennsylvania, just north of the Maryland border. It is underlain largely by serpentinite bedrock of the Baltimore Mafic Complex (BMC). This complex of ultramafic and associated gabbroic rocks is believed to be a remnant from the roots of an island arc complex. Evidence supporting this origin includes the oxide composition of chromite in ores, the relative abundances of platinum group elements in chromite ore composites, and the association with Bald Friar Metabasalt backarc and "Conowingo Creek metabasalt" forearc basalts nearby. Various portions formed at 490 +/- 20 Ma above a southeast-dipping subduction zone. This zone incorporated detritus from the margin of the Laurentian continent and nearby microcontinents, as well as ultramafic oceanic material from the floor of the Iapetus Ocean isolated from the mantle during the older, 735-Ma period of rifting. Both the backarc and forearc basalts are found as masses along with ultramafic fragments from the BMC proper in an ophiolite mélange that formed in the trench that developed on the northwestern side of the arc complex as the Octoraro basin was tectonically closed. Traditionally, the Iapetus Ocean is considered to have closed during the Taconic Orogeny, circa 450 Ma, by obduction of the island arc and mélange onto the margin of Laurentia. The obduction of the BMC was likely aided by the presence of zones of low competence developed within steatized ultramafic fragments within the mélange-bearing trench. The BMC was subjected to a high temperature, low pressure metamorphism circa 440 Ma. This metamorphism and associated igneous activity appears to have been mainly the result of crustal thinning following the Taconic orogeny and, to a lesser extent, loading from the orogeny. The serpentinites within the Goat Hill tract have widely varying compositions as a result of the sequence of minerals that crystallized from the BMC when it was still part of the ocean floor (forsterite to orthopyroxene to clinopyroxene to pyroxenes plus plagioclase), later metamorphic events after it had been obducted onto the continental margin, and variable leaching by recent meteoric water.
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INTRODUCTION

The Goat Hill Serpentine Barrens are located in extreme southwestern Chester County, just north of the Mason and Dixon Line, which defines the border between Pennsylvania and Maryland, (Figure 1). The Goat Hill tract is underlain largely by the Baltimore Mafic Complex (BMC), a fragment of an ancient island arc and related oceanic crust.

Long-term management of Goat Hill is the responsibility of the Pennsylvania Bureau of Forestry, part of the Department of Conservation and Natural Resources (DCNR). The nurturing of rare plant habitat is one of their primary goals for Goat Hill. Typical of many serpentinite tracts in eastern North America, Goat Hill contains a variety of unusual microenvironments, not found elsewhere in the region, that are characterized by combinations of serpentinite bedrock

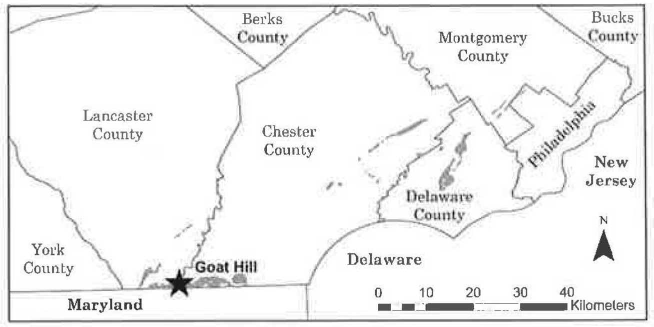

Figure 1. Location of the Goat Hill Serpentine Barrens, Chester County, Pennsylvania. Shaded areas indicate serpentinites of ultramafic origin.

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chemistry, albedo, and drainage. At the request of the another DCNR agency, the Bureau of Forestry, the Bureau of Topographic and Geologic Survey conducted a detailed study of Goat Hill to provide a better understanding of its geology, which is essential for wise stewardship of the area. This paper focuses primarily on recent geologic and geochemical data collected on the BMC and presents and interprets new data obtained for this study. A companion paper, "Bedrock Composition of the Goat Hill Serpentine Barrens and a Proposed 'Serpentine Factor' for Predicting Floral Response" (Smith and Barnes 2008), is also a result of that request. In addition to presenting bedrock-composition data specific to Goat Hill and indicating what it suggests for plant habitat, Smith and Barnes (2008) use that data to propose a new, general formula for calculating a quantitative serpentine factor that, it is believed, will predict the suitability of serpentine flora habitat prior to restoration efforts.

Smith and Barnes (1999) had earlier described the geology of Nottingham County Park (NCP), 4 km to the east-northeast of Goat Hill, which is also largely underlain by serpentinite. The NCP paper benefited from the reviews of many, but especially the former Professor Robert R. Brooks, D.Sc., Massey University, New Zealand, who provided the authors his insight into the unique serpentinite environment. Both Smith and Barnes began their geological careers at the Pennsylvania Survey under the direction of Dr. Davis M. Lapham, who had studied and mapped the Baltimore Mafic Complex to the west of Goat Hill. Indirectly, Lapham; Dr. Edgar T. Wherry, a former mineralogist/botanist acquaintance of Smith; and Prof. Brooks helped inspire this study.

STUDY AREA

The Goat Hill Serpentine Barrens make up an irregularly shaped 244-hectare tract in West Nottingham Township in extreme southwestern Chester County, Pennsylvania, in the Rising Sun 7½' quadrangle. It is owned by the Commonwealth of Pennsylvania and managed by the Bureau of Forestry of the Department of Conservation and Natural Resources. The boundary of Pennsylvania and Maryland, the Mason and Dixon Line, forms the southern boundary of much of the tract; a meander of Octoraro Creek forms a segment of the northern boundary of the tract with Lancaster County. This area lies in the Piedmont physiographic province.

Geology, Ages, and History

Geologically, most of the Goat Hill tract is underlain by ultramafic rocks of the Baltimore Mafic Complex (BMC). More complete descriptions of the BMC are given by McKague (1964) and Hanon and Sinha (1989). The portion of the BMC along the Mason and Dixon Line that produced chromite in commercial quantities during the nineteenth century is typically known as the State Line District when discussing economic geology.

The BMC likely formed in the portion of the Neoproterozoic to Ordovician Iapetus Ocean known as the Octoraro Sea, which was located east of the shore of the continent of Laurentia (using present coordinates) but inboard of the Brandywine and Baltimore microcontinent massifs of Faill (1997). The BMC includes portions of the ocean floor and imbedded island arcs of the former Iapetus Ocean that were obducted (thrust northwest, present coordinates) up over the edge of the margin of the Laurentian continent when that ocean closed up. The obducted material likely also included ocean floor basalts, especially those generated in a back arc setting such as the Bald Friar Metabasalt and Kennett Square Amphibolite (Smith and Barnes 1994; Smith 2004 and 2006), the island arc James Run Formation as defined by Higgins (1977), and the informally defined, boninitic affinity "Conowingo Creek metabasalt" (Smith and Barnes 1994). To the east along the Delaware-Pennsylvania border, the plutonic and volcanic island arc Wilmington Complex (WC) (Armstrong et al. 2001, Bosbyshell 2003, and Aleinikoff et al. 2006) was also obducted.

On the north, the BMC is bounded by the Sykesville Formation. It was originally mapped in Cecil County, Maryland, by Higgins and Conant (1986). Smith and Barnes (1994 p. 62) recognized the Sykesville Formation as an ophiolite mélange containing clasts of the Bald Friar Metabasalt and ultramafic fragments. Many of the latter were steatized and attenuated by obduction and thrusting. The Sykesville Formation of Conant and Higgins is equivalent to the Morgan Run Arm Formation, Carroll County, Maryland, of Muller et al. (1989). Faill and Smith (in review) extended the Sykesville Formation ophiolite mélange into Pennsylvania to include ultramafic clasts up to kilometer-scale as well as other exotic lithologies.

It is traditionally accepted that the floor of Iapetus began forming not long after the transition from Catoctin continental rift (dated at 564 +/-9 Ma for Catoctin Metarhyolite, Aleinikoff et al. 1995) to oceanic drift, with a concomitant shift from continental rifting basalt to oceanic drift facies basalt (Smith and Barnes 1994, and Smith and Barnes 2004, Table 3). However, as addressed in the Results and Discussion section below, there is evidence that the mantle component of the rock that formed the floor of Iapetus was isolated from the deeper mantle much earlier in the previous cycle of rifting that is associated with the 735- to 702-Ma Robertson River Igneous Suite (Tollo and Aleinikoff 1996). Until recently, closure of Iapetus has been included in the Taconic orogeny, typically dated at ~458 Ma at the latitude of Pennsylvania. However, new age estimates of Taconic metamorphism and hydrothermal mineralization are available and discussed below. Traditionally, the next period of orogeny in this portion of Pennsylvania was the Alleghanian at ~275 Ma. Again, new data have been obtained indicating a period of widespread crustal thinning, igneous activity, and thermal metamorphism at ~435 Ma, well between the classic Taconic and Alleghanian orogenies.

The oldest proposed dates for the BMC include slightly discordant zircon U-Pb dates of 510 Ma and 512 Ma for gemmy, zirconoid habit zircons (A. A. Drake, pers. comm., 1978, for zircons he and Smith collected separately), but it is now known that the BMC incorporated Grenvillian detritus from Laurentia and/or one of the Brandywine massif micro-continents. Thus, a minor, but much older, Grenvillian-age core in those zircons cannot be ruled out. Sinha et al. (1997) reported 489 +/- 7 Ma based on 4 scattered points in a U-Pb discordia diagram for zircons from plagiogranite plus quartz gabbro. By itself, this might not be convincing, but it agrees remarkably well with estimates by Shaw and Wasserburg (1984). They reported ~490 +/- 20 Ma based on two distinct Sm-Nd evolution diagrams for minerals separated from two gabbros. Thus, much of the main stage of the BMC developed at 489 +/- 7 Ma, as reported by Sinha et al. (1997).

The Wilmington Complex (WC), a second island arc complex and likely a geologic cousin to the BMC, is located 50 km to the east of the BMC. Using a Sensitive High-Resolution Ion Microprobe (SHRIMP) to study complexly zoned zircons, Aleinikoff et al. (2006) dated six igneous units in the WC. They obtained dates of 476 +/- 6 Ma, 476 +/- 4, 482 +/-4, 481 +/- 4, 483 +/- 7, and 476 +/- 8 Ma for intrusive and metavolcanic units. Thus, accumulating evidence suggests that the main stage of formation of the WC occurred over the range 475–485 Ma (Aleinikoff et al. 2006). Such extended igneous activity does not seem unreasonable for the development of a complex island arc such as the WC and supports the 489 +/- 7 Ma age for the BMC (Sinha et al. 1999).

Grauert and Wagner (1975) reported an ~440-Ma granulite facies metamorphism of the WC based on nearly concordant analyses of zircon fractions from a banded gneiss. Foland and Mussig (1978) confirmed this ~440-Ma granulite-grade metamorphism with Rb-Sr analyses of four mineral separates from the WC plus a whole-rock aliquot that did not fit their igneous isochron. Most recently, Aleinikoff et al. (2006) reported that the WC was cut by the Arden Pluton at 434 +/- 5 Ma. Thermal metamorphism associated with this igneous activity yielded zircon overgrowth dates of 428 +/- 4 and 432 +/- 6 Ma and monazite dates of 429 +/- 2 and 426 +/- 3 Ma. Taken together, these dates strongly support a metamorphic stage at ~435 Ma for the WC area.

Recently it has become apparent that the continental crust in the mid-Atlantic states was appreciably thinned at 435 +/- 10 Ma, resulting in intrusion and extrusion of unusually hot magmas, as well as the metamorphism noted above. Smith (2006) proposed the term Silurian Thermal and Magmatic event (STM) for this tectonic phase.

One result of STM-thinned continental crust is the extrusion of mantle-derived 433 +/- 3 Ma Sword Mountain Olivine Melilitite (*OM*) of the Clear Spring Volcanic Suite located 120 km to the west of Goat Hill. There, fine-grained groundmass phlogopite from the *OM* yielded 432.6 +/- 2.4 Ma by incremental heating $^{39}Ar/^{40}Ar$ measurements. Rb-Sr analysis of the same phlogopite and whole rock aliquots yielded an indistinguishable concordant age of 434 +/- 3 Ma (analyses by K. A. Foland in Smith et al. 2004). Both types of radiometric dates confirmed the stratigraphic age. A related result of crustal thinning is the Beemerville Igneous Complex of northwestern New Jersey, which yielded a K/Ar date of 445 +/- 22 Ma (herein corrected K decay-constant) and Rb-Sr dates of 436 +/- 41 Ma and 424 +/- 20 Ma, all on biotites (Zartman et al. 1967). An example of thinned crust to the east of Goat Hill is the 434 +/- 5 Ma intrusive, igneous phase of the Arden Pluton which intruded the older WC (Aleinikoff et al. 2006).

Trujillo and Sinha (2004) document mantle-derived gabbros to diorites in extensional settings to the south in Virginia, providing a sense of the magnitude and extent of the STM crustal thinning at 435 +/- 10 Ma. They constrained the ages of four such mafic complexes to between 434 and 431 Ma using U/Pb zircon ages. To the north, Karabinos et al. (2004) dated the Beckett Quarry volcanic arc granite in Connecticut at 432 +/- 3 Ma using $^{206}Pb/^{238}U$ dating of zircons and propose a relationship to extensional tectonics and the opening of the Connecticut Valley trough.

Thus, dates for island arc affinity lithologies pre-date the classical Taconic orogeny by 30 Ma and dates of extensional igneous and metamorphic activity post-date it by 20 Ma. As a consequence, these data require downgrading the impact of the Taconic Orogeny, *sensu stricto*, in southeastern Pennsylvania. This is consistent with the SHRIMP zircon dates of Gray and Zeitler (1997), who found an absence of Taconian-age zircons in Lower Silurian Shawangunk conglomerate. In the Pennsylvanian-age Pottsville conglomerate, they found that "The main cluster of zircons in Pottsville clasts is between 400 and 450 Ma. These dates fall precisely between currently accepted limits of Taconic (~440 - 470 Ma) or Acadian (~365–405 Ma)..." (Gray and Zeitler, 1997, p. 157). However, they do, within error limits, seem to fit the 435 +/- 10 Ma STM of Smith (2006).

It is possible that tectonism and volcanism associated with the end of Helderberg carbonate deposition (Smith et al. 1988) corresponds to the final ophiolite obduction. Tucker et al. (1998) date zircons in Bald Hill Bentonite A, Cherry Valley, New York, from near the top of the Helderberg Group, at approximately 417.6 +/- 1 Ma weighted average $^{207}Pb/^{206}Pb$ age. (More accurate and precise dating of Bald Hill Bentonite C from the type locality, Bald Hill, Pennsylvania, is discussed below under Results and Discussion.) Although no volcanic source area has been identified, it is presently widely assumed that the Bald Hill Bentonites are the result of island arc volcanism associated with closure of an ocean or large sea along a destructive plate margin. If so, they might represent final closure of Iapetus. These young age interpretations are similar to those proposed for the Newfoundland Appalachians by Cawood et al. (2001).

Despite the fact that most available dates of the BMC *sensu stricto* have complexities, an overall range of dates for the BMC and presently recognized island arcs in Iapetus of 489 +/- 7 Ma (Sinha et al., 1997) seems reasonable. For possible evidence for older relicts of Iapetus, see Results and Discussion. The younger 435 +/- 10 Ma dates associated with widespread STM crustal thinning and metamorphism are quite widespread. Gravitational collapse and ophiolite pullback are inadequate mechanisms to explain this widespread distribution. Plate thinning caused by a temporary reversal of plate motions seems to be required.

Petrography and Mineralogy of Serpentinite

As described by McKague (1964) and later by Hanan and Sinha (1989), the BMC exposed along Octoraro Creek and at other exposures just west of the Goat Hill tract generally consist of successively higher magmatic, cumulate layers of forsterite, orthopyroxenes, clinopyroxenes, calcic plagioclase, and mixtures thereof overlain by gabbro. The first three of these minerals have been largely metamorphosed and/or altered by low-temperature reactions to serpentine-group minerals. These minerals superficially resemble one another despite distinct differences in MgO and Ni, high concentrations of which should be favorable to serpentine-endemic flora, and CaO, K_2O, and P_2O_5, high values of which are unfavorable (see companion paper Smith and Barnes 2008).

Based on 115,000 points identified and counted in thin sections, McKague (1964) found that the serpentinite portion of the BMC just to the west of the Goat Hill tract contains 80.81% serpentine group minerals, 4.64 olivine, 5.62 opaque minerals (such as magnetite and sulfides, especially heazlewoodite), 1.22 chromite, 0.98 talc, 2.48 carbonate minerals, 1.41 brucite, and 2.85% other minerals such as chlorite. X-ray diffractometer scans of 15 serpentinite bedrock composites from Chester County's Nottingham County Park (NCP) (Smith and Barnes 1998), 3 samples from the tract northeast of NCP owned by the Nature Conservancy (TNC), and 30 from the Goat Hill tract suggest that the most abundant serpentine group mineral in the Goat Hill tract is antigorite; that from Nottingham County Park to the northeast is lizardite.

Alteration at lower temperatures is also indicated. Wenner and Taylor (1974) included samples from just west of the Goat Hill tract in their study of deuterium and oxygen isotopes. From the oxygen isotope ratios of coexisting antigorite and magnetite in two samples from the Cedar Hill Quarry, 1.4 km west of Goat Hill, they estimated temperatures of serpentinization of magmatic olivine to antigorite of 300 +/- 15°C. This and their deuterium isotope data led them to propose a metamorphic origin for the serpentine minerals by non-meteoric waters. From deuterium and oxygen isotope studies of lizardite +/- chrysotile from many other locations, they conclude that lizardite typically forms at around 100°C from meteoric waters. Indeed, they found a good positive correlation of calculated deuterium isotope ratios to that of modern day meteoric water which, in turn, correlates with latitude. Thus, the major bedrock mineral at Goat Hill is metamorphic antigorite whereas the lower temperature alteration mineral lizardite is predominant at NCP. It is interpreted herein that the lizardite may be a younger overprint, but this is far from certain.

Alternately, lizardite formation from olivine may be an ongoing process. If so, at NCP there may be 1) unrelieved stresses accompanying the volume increases associated with present day serpentinization of olivine and orthopyroxene by meteoric water to lizardite, and 2) trace-free hydrogen and accompanying reduction in the seeps draining such areas. Reduction of trace Ni and Fe to native metal cannot be ruled out and, where carbon is present, trace abiotic methane might be produced. Such a reduction process might have occurred at the base of the Oakryn serpentine body, 7 km northwest of Goat Hill, where Smith and R. T. Faill verified graphite plus talc on the south side of Soapstone Hill Road (October 4, 1994), approximately where D. M. Lapham recorded its presence in his notes in the 1960's. Graphite, indicative of such past reduction, may also be present at the base of the serpentine on the southwest side of Pine Run, just southeast of its confluence with Octoraro Creek.

Limited amounts of three other ophiolitic aspects of the BMC occur not far from Goat Hill. Thin dikes of grossly basaltic composition cut serpentinite in Nottingham County Park (Smith and Barnes 2004), a bed of pillow basalt of Bald Friar Metabasalt (Smith 2004) occurs near the type locality, and the boninitic affinity "Conowingo Creek metabasalt" (Smith and Barnes 1994) occurs to the west.

The originally igneous forsterite layers, now largely metamorphosed to antigorite at Goat Hill, contain disseminated chromite and locally pods of chromite, some of which were of commercial importance (Pearre and Heyl 1960). Those deposits in the portion of the BMC that includes the Goat Hill tract constitute the State Line Chromite District. Pearre and Heyl (1960) reported that the Hillside Mine, located in the Goat Hill tract, probably produced 15,000 tons of chromite prior to 1900. Gorrecht (1935), in a somewhat promotional document, reported a trench to the south of Octoraro Creek and almost due south of the Wood Mine (a major chromite mine 0.3 km north of the Goat Hill tract) that extended from the top of the hill almost to the bottom. He believed that this trench yielded an "immense" amount of residual chromite, but that it was completely worked out. The trench is presumably located in the Goat Hill tract, but it was not observed in traverses during the present study. (Note that large deposits of a dense, residual mineral such as chromite are not likely to accumulate or remain on steep slopes.) McIntosh and Mosier (1948) reported 700 m of trenching by the U.S. Bureau of Mines that revealed a "show of chrome" on the Yinger Farm, 0.6 km west of the Wood Mine.

Because of its usefulness in recognizing areas favorable for endemic serpentinite, chromite is further discussed in the companion paper, Smith and Barnes (2008). Because of the potential for extreme microenvironments, magnesite deposits are also discussed in that paper.

In addition to chromite and magnesiochromite, several other metallic minerals are found in serpentinite near Goat Hill. Smith and Spear (1980a) reported primary heazlewoodite, Ni_3S_2, (0.04 to 0.07% Fe, 0.10 to 0.12 Co, 71.49 to 71.58 Ni, 0.26 to 0.32 Cu, and 28.31 to 28.49% S by quantitative electron microprobe) typically in composite grains with pentlandite and slightly later millerite from the Cedar Hill Quarry, 1.4 km to the west. Smith and Spear (1980b) reported maucherite, $Ni_{11}As_8$, (52.30 to 53.87% Ni, 0.15 to 0.29 Cu, 0.07 to 0.19 Co, 0.61 to 1.36 S, and 45.44 to 46.31% As by quantitative electron microprobe) and millerite, NiS, as rims on pentlandite, $(Fe, Ni)_9S_8$, from the Wood Chromite Mine. These Wood Mine minerals were confirmed by standardless SEM/EDS in additional samples collected during the present study (Table 1).

Table 1. Standardless SEM/EDS analyses of some nickel sulfide and nickel arsenide minerals (two maucherites, three millerites, and one pentlandite) from the Wood Chromite Mine, just across Octoraro Creek to the north of the Goat Hill Serpentine Barrens.

Maucherite		Millerite		Pentlandite	
Ni	51.2%	Ni	62.4%	Ni	36.2%
Fe	0.8	Fe	0.8	Fe	28.5
As	40.1	S	36.8	S	35.4
S	7.8				
		Ni	64.7		
Ni	52.3	Fe	0.4		
Fe	ND	S	34.9		
As	46.6				
S	1.1	Ni	62.4		
		Fe	ND		
		S	37.6		

ND: Not detected.

As noted, the BMC is believed to be internally layered by igneous cumulates that fractionated during cooling. Both these internal layers and the lower tectonic contact upon which the BMC was obducted up onto Laurentia are believed to dip to the south but may not be strictly parallel. Poor exposures just southeast of the confluence of Pine Run and Octoraro Creek suggest a strike of approximately N75°E in that area and a steep dip to the south. Chromite schlieren in Nottingham County Park, 4.1 km to the east-southeast, trend N27°E and dip 69°SE. Strike of bedrock is perhaps best generalized from poor, distant exposures of the northern serpentine contact in the Goat Hill Tract. These yield a strike of ~N80°E for the Goat Hill area, but the strike of the contact is somewhat undulatory.

Because serpentinization of forsterite and pyroxenite tends to obliterate all but a relict parting in the latter, such data were, until Smith and Barnes (2008), about all the geologic data available to project bedrock chemical compositions in the area. Fortunately, greenbrier (*Smilax*) does not seem to compete well beyond areas underlain by fresh serpentinite. This observation can be used to map faulted offsets in the southern limit of serpentinite with the overlying gabbro. A few such areas are readily apparent to the west of the crest of Goat Hill and relatively near to the Mason and Dixon Line. These offsets tend to correlate with north-south trending drainage gullies, adding credence to this fault offset hypothesis.

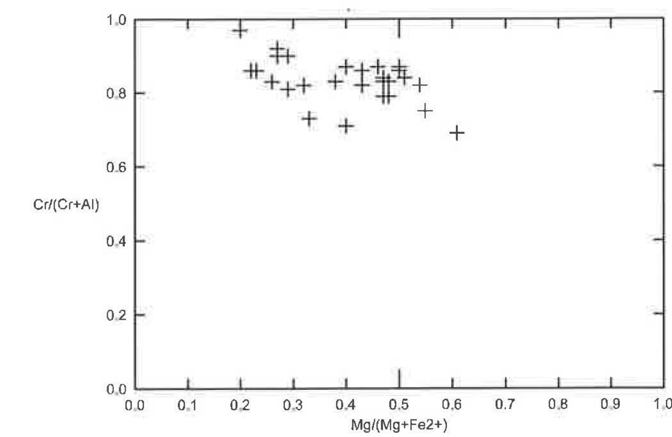

Figure 2. Plot of Cr/(Cr+Al) versus $Mg/(Mg+Fe^{2+})$ for chromite samples collected in the State Line District which encompasses Goat Hill. The data were collected via standardless energy-dispersive X-ray spectrometry. The values used for Fe^{2+} assume that iron is present in the ratio of 0.78 Fe^{2+}/0.22 Fe^{3+}.

RESULTS AND DISCUSSION

Three previously "lost" chromite mines in the Goat Hill Serpentine Barrens were relocated during the present study. Of these, some historical data were available for the Hillside Mine (Pearre and Heyl 1960), but the history of the other shafts, herein named GH 33 and GH 34, is unknown. Three polished sections were prepared from Hillside Mine chromite ore, two each from GH 33 and 34, and 22 from samples representing 12 other deposits in the State Line District. Several of the 22 samples were selected from the senior author's mineral collection, begun with help from mineralogist-botanist *extraordinaire*, Edgar T. Wherry, at the Wood's Chrome Mine circa 1955. Multiple points on each polished section were then analyzed by SEM/EDS; the results are presented in Table 2.

To interpret the origin of chromite deposits, the relationship Cr/(Cr + Al)/ $Mg/(Mg + Fe^{2+})$ was plotted (Figure 2), as per the method of Stowe (1994). The high and relatively constant Cr/(Cr + Al), broad spread of $Mg/(Mg + Fe^{2+})$, and low TiO_2 (Table 2) suggest that all of the chromites originated in the lower cumulate zone of a magma chamber. The high initial $Mg/(Mg + Fe^{2+})$ suggests accumulation in a back arc or, more likely in this case, subarc tectonic setting. This is consistent with the volcanic arc origin of the BMC advanced by Sinha et al. (1997) using different criteria.

Subarc lower cumulate zones, in and of themselves, do not explain how the present podform nature of the better, commercial deposits in the BMC originated. It is proposed that the change from stratiform chromite layers in the lower portions of a subarc magma chamber to pods was accomplished by inward folding and collapse of the denser chromite layers into hot forsterite crystal mush. These folded chromite layers sank down as negative diapirs into less dense forsterite layers until stopped by the earlier and cooler (and therefore more solid) forsterite cumulate layers near the base of the BMC.

Table 2. Standardless SEM/EDS analyses of chromites from the State Line Chromite District, which includes Goat Hill. Samples having a "GH" prefix were collected at the Goat Hill tract. Those having an "NCP" suffix were collected within Nottingham County Park. Wood Mine is in a meander of Octoraro Creek, bordered on three sides by the Goat Hill tract. All analyses are given in percent.

Sample	MgO	Al_2O_3	TiO_2	Cr_2O_3	$Fe_2O_3^*$	FeO^*	NiO	TOTAL	Latitude (N)	Longitude (W)
Line Pit A	14.4	10.8	0.1	58.2	3.6	11.4	0.0	98.6	39°43'16"	76°10'18"
Line Pit B	13.0	11.7	0.0	58.0	4.1	13.2	0.0	100.0		
Line Pit C	14.3	11.7	0.0	57.8	3.5	11.2	0.0	98.5		
Graybeal Prospect	13.0	10.6	0.0	54.5	4.5	14.2	0.0	96.8	39°43'43"	76°09'46"
Red Pit A	14.8	13.9	0.0	54.7	3.5	11.3	0.0	98.3	39°43'26"	76°09'44"
Red Pit B	13.7	10.6	0.0	56.6	3.9	12.6	0.0	97.5		
Red Pit C	16.2	14.6	0.0	50.7	3.4	10.8	0.0	95.6		
Wet Pit	14.0	13.2	0.0	53.8	3.8	12.3	0.0	97.2	39°43'28"	76°09'42"
North Rock Springs	13.9	14.6	0.0	54.4	3.9	12.4	0.0	99.3	39°43'37"	76°09'26"
Horseshoe prospect	9.7	14.9	0.0	48.6	5.7	18.1	0.0	96.9	39°43'12"	76°07'47"
Newbold Mine	14.3	14.5	0.0	54.0	3.8	12.3	0.0	99.0	39°43'42"	76°07'15"
GH 33 A	15.3	16.6	0.0	47.1	4.0	12.6	0.0	95.6	39°43'47"	76°06'54"
GH 33 B	15.1	17.1	0.0	49.2	4.2	13.2	0.0	98.8		
GH 34 A	13.6	13.5	0.0	48.3	4.4	14.0	0.0	93.8	39°43'45"	76°06'51"
GH 34 B	12.9	14.0	0.0	52.2	5.2	16.6	0.0	100.8		
GH Hillside Mine A	16.2	20.3	0.0	46.7	3.3	10.4	0.0	96.9	39°43'42"	76°06'44"
GH Hillside Mine B	7.9	2.2	0.0	53.1	7.6	24.3	0.0	95.2		
GH Hillside Mine C	18.4	25.6	0.0	43.3	2.8	9.0	0.0	99.0		
Wood Mine A	9.5	6.5	0.2	54.6	6.4	20.4	0.3	98.0	39°43'51"	76°06'22"
Wood Mine B	10.0	7.8	0.1	52.0	6.7	21.4	0.4	98.4		
Wood Mine C	10.6	7.4	0.0	53.6	6.2	19.7	0.0	97.4		
Kirk Mine, NCP 1	8.1	10.4	0.0	51.3	6.7	21.4	0.0	98.1	39°43'52"	76°03'03"
Kirk Mine, NCP 2	9.2	12.1	0.0	47.1	6.2	19.8	0.0	94.4		
Kirk Mine, NCP 6	7.9	10.8	0.0	51.5	6.9	21.9	0.0	98.9		
Cr 5, NCP	11.6	19.9	0.0	41.8	5.8	18.6	0.0	97.6	39°44'11"	76°02'55"
Cr 8, NCP	10.6	14.4	0.0	49.9	5.5	17.6	0.0	98.0	39°43'43"	76°02'35"
Cr 11, NCP	13.1	21.9	0.0	40.9	4.8	15.3	0.0	96.0	39°44'03"	76°02'35"

*Iron assumed to be in the ratio of $0.78Fe^{2+}/0.22Fe^{3+}$.

Both the senior author's negative diapir theory and the subarc interpretation were confirmed by Clive W. Stowe, using some early chromite data (C. Oliver Ingamells, analyst, Mineral Constitution Laboratory, The Pennsylvania State University) presented initially in McKague (1964), but more readily available in Table 17 of Smith (1978). Stowe (pers. comm., 9/29/1994) noted that density driven collapse was possible and that the chromite signatures supported a subarc origin for the BMC. Stowe also noted a marked similarity, except for having a slightly lower and slightly wider range of Cr/(Cr + Al), to the compositions of chromite from Thetford, Quebec, Canada. This similarity between Thetford and the BMC comes as no surprise based on the noted extreme similarity (Smith 2006, p. 253) of Caldwell Group 1b pillow basalts of the Thetford area (Bédard and Stevenson 1999) and the Bald Friar Metabasalt of Pennsylvania and Maryland (Smith 2006).

Analyses of platinum group elements (PGE) in chromite composites were obtained from the three mines relocated within the Goat Hill Serpentine Barrens (Table 3). Each of the chromite composites was high-graded by preferentially collecting field-visible chromite. Considering the normal high variability in PGE data, these results are relatively similar to chromite composites Cr-5, -8, and -11 of NCP (Smith and Barnes 1998, p. 31). They are also consistent with formation of the chromite and host BMC in ophiolite beneath an island arc (Prichard et al. 1994). The three composites from Goat Hill lost much of the more S-loving Pt and especially Pd after encountering a S-rich component. In the case of Goat Hill, some of this Pt and Pd may have been redeposited in stratigraphically higher samples such as GH 21 and GH 30, which may have been derived from metapyroxenites based on their somewhat lower Cr and Ni. In the case of Nottingham County Park, Cr-7 and Cr-16 seem to have

Table 3. Analyses of chromite composites from the Goat Hill serpentinite tract, Chester County, Pa. Each sample is a composite of 15 pieces, each 4 to 6 cm, high-graded with respect to chromite from lean dumps. Cr_2O_3 analyses by X-ray fluorescence. Au and platinum-group elements by NiS fire assay and inductively coupled plasma mass spectroscopy (ICP/MS). All analyses by Activation Laboratories, Ltd.

	Cr_2O_3 percent	Au ppb	Ru ppb	Rh ppb	Pd ppb	Re ppb	Os ppb	Ir ppb	Pt ppb
GH-33, chromite dump	20.9	49	237	136	3	<1	21	82	228
Hillside Chromite Mine	16.8	21	45	103	1	<1	14	25	191
GH-34 Twin chromite dump	18.4	51	123	138	3	<1	20	93	263

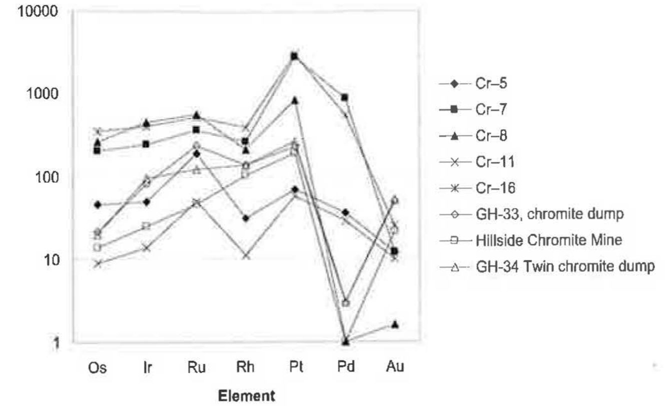

Figure 3. Mantle normalized (McDonough and Sun 1995) platinum group elements (PGE) plus Au determined by NiS fire assay-ICPMS on the highgraded composites of chromite listed in Table 3. GH-33, GH-34, and Hillside mine samples from the Goat Hill Serpentine Barrens. Cr- series samples from Nottingham County Park, approximately 4 km to the east-northeast.

avoided early contact with sulfur and concomitant loss of Pt and Pd. Mantle-normalized PGE plus Au for both Goat Hill and NCP chromite composites are shown in Figure 3.

Age of the Baltimore Mafic Complex.

Arc volcanics similar to the BMC that have formed near a continental margin, near microcontinents, or near both, are typically a remelted blend of ultramafic mantle material and continental detritus. Nd isotopes (Shaw and Wasserburg 1984), Pb and Sr isotopes (Sinha et al. 1997), and Hf isotopes (Rooney 2006) support a magma composition having a continental influence. This is especially true if the arc is being obducted onto a continental margin. In an effort to date the isolation of the ultramafic material from the mantle, microscopic grains of PGE osmium alloys were sought. A microscopic OsIrRu grain (52.3 % Os, 38.4 Ir, 7.9 Ru, 1.2 Fe, and 0.2 % Ni by standardless SEM/EDS) was sent to the University of Arizona for $^{187}Os/^{188}Os$ analysis by Ryan Mathur of Juniata College. It yielded an age of isolation from the deep mantle of 735 Ma (assumed mantle Re/Os = 0.42 and measured $^{187}Os/^{188}Os = 0.1296$ +/- 0.05 %). This corresponds with a previously known mantle-separation age of 735 Ma based on a model T_{Nd} age for the Sword Mountain Olivine Melilitite, which was extruded at 433 +/- 3 Ma (K. A. Foland in Smith et al. 2004). (T_{Nd} represents the calculated time when Nd ceased to be in equilibrium with the mantle, i.e., the time when its residence in the crust began. It is a "model" age because of a required assumption that the source area in the mantle had been previously been "depleted" similar to those areas of the mantle capable of producing mid-ocean ridge basaltic melts.) This 735 Ma also happens to be the approximate age of the Robertson River Igneous Suite in Virginia (Tollo and Aleinikoff 1996).

The 735 Ma age for separation of the ultramafic component in the BMC from the mantle is important because it also helps confirm a very early age for the initiation of the Iapetus Ocean, the early products of which are generally not well preserved. It is relevant to the present study because it is the closure of the Iapetus Ocean that ultimately provided the serpentine to the BMC barrens. Thus, the history of the BMC spans a complete Wilson cycle from the initiation to closure of the Iapetus Ocean.

As noted in the Study Area section above, A. K. Sinha et al. (1997) determined that the igneous age for the BMC (intrusion into the floor of Iapetus) is 489 +/- 7 Ma. Below, we attempt to complete the dating history by determining: 1) when the Taconic mountains were built in this area, 2) when the obducted BMC was "stitched" to the continent of Laurentia, and 3) the "model" age of the Laurentian continental material being subducted beneath the island arc that was blended with the mantle material to ultimately yield the serpentine.

1) Taconic Mountains in this region: A 2-mm gemmy monazite in galena was collected by the senior author from the Pequea Silver Mine A area, approximately 30 km NW of Goat Hill. Wise et al. (2007) report a 450 +/- 4 Ma date for this monazite based on ion microprobe Th-U-total Pb. Fluid inclusions in quartz associated with the monazite and galena (measured by C. M. Onasch, Bowling Green State University, reported by Wise et al. 2007) indicated heating by hydrothermal fluids to 215–265°C at this time. Presumably, these fluids were being expelled from the interior of the new mountains built as a result of the Taconic Orogeny. This is probably the orogeny that obducted the island arc from the floor of the Iapetus Ocean onto the edge of the continent Laurentia. Presently, it cannot be ruled out that the oceanic material first overrode the Brandywine massifs. The map pattern (Berg et al. 1980) is permissive of such a bumpy route.

2) Stitching of the Baltimore Mafic Complex to the continent of Laurentia: A remobilized refractory Fe-Cr-Ti-Zr oxide forming an "iron ore" (Table 4) was found by the senior author and S. W. Berkheiser at the Reading Iron Company Mine in the serpentine barrens of Fulton Township, Lancaster County, 6 km to the west of Goat Hill. Here, serpentinization and deformation have conspired to camouflage contacts and the only "iron ore" observed was on the dumps. However, this Fe-Cr-Ti-Zr ore is similar in stratigraphic level and appearance to many oxide pods elsewhere in the district that are richer in Cr. Ultimately, it might represent a refractory residue that for many elements is chemically the opposite of an island arc gabbro or basalt. As such, it might have initially formed as cumulate layer similar to the chromites, but at a higher level, after which it formed a dense, negative diapir near the base of a hot, developing island arc. There, it gradually went through a series of cooling-induced exsolutions to finally yield late-crystallizing ilmenite-baddeleyite globules. The 7.2 mol percent ulvöspinel in the magnetite and 87.6 mol percent ilmenite in coexisting ilmenite-hematite solid solution (Table 5) indicate that these Fe-Ti oxides were last in equilibrium with one another at 665 +/- 20°C and at an fO_2 (availability of oxygen) of $10^{-15.2 +/- 0.2}$, based on the analyses in Table 4 and the calibration of Spencer and Lindsley (1981).

Table 4. Composition of baddeleyite-bearing "iron ore" enriched in Fe-Cr-Ti-Zr, Reading Iron Mine, Fulton Township, Lancaster County, Pa. Sample RIMTE 11/20/03 is a composite of ~1.0-cm-thick slabs from the cores of five different pieces of ore. Analyses by Activation Laboratories, Ltd.

Major and minor oxides (%)		Trace elements (ppm)				Rare Earth elements (ppm)	
SiO_2	4.77	Ag	30	Rb	3	La	1.29
Al_2O_3	0.91	As	40	S	<10	Ce	1.71
Fe_2O_3	57.54	Ba	26	Sb	2.9	Pr	0.24
MnO	0.78	Co	376	Sc	23.8	Nd	0.82
MgO	9.26	Cu	77	Se	7.4	Sm	0.16
CaO	0.04	Cr	22600	Sn	2	Eu	0.04
Na_2O	0.05	Cs	0.2	Sr	2	Gd	0.18
K_2O	0.04	Ga	5	Ta	10.2	Tb	0.05
TiO_2	24.06	Hf	232	Th	1.34	Dy	0.49
P_2O_5	<0.02	Mo	<2	U	4.69	Ho	0.18
		Nb	123	V	1614	Er	0.93
		Ni	3745	Y	25	Tm	0.25
		Pb	19	Zn	363	Yb	2.41
				Zr	9771	Lu	0.58

Isotopic Data (present day)	Result	Error	Derived From
$^{143}Nd/^{144}Nd$	0.512071	+/-9 (2 sigma)	^{147}Sm
$^{206}Pb/^{204}Pb$	21.643*	0.1%	^{238}U
$^{207}Pb/^{204}Pb$	15.794*	0.15%	^{235}U
$^{208}Pb/^{204}Pb$	38.367	0.2%	^{232}Th

* These high values correspond to HIMU, variously listed as either Highly Enriched Mantle Uranium or high μ ($^{238}U/^{206}Pb$), a grouping of Pb isotope compositions in mafic rocks corresponding to remelted, subducted oceanic crust which lost its Pb via the hydrothermal fluids expelled from the subducting, dehydrating slab, but which retained its U as the immobile, insoluble U^{4+} ion in a reduced environment. The HIMU signature further supports the island arc affinity of the Baltimore Mafic Complex.

Table 5. Standardless SEM/EDS analyses, in percent, of two distinct areas on polished slice R.I. 1 from the Reading Iron Mine, Fulton Township, Lancaster County, Pa. "Ilm.-Hem." is a 20×20-micrometer area of ilmenite-hematite solid solution that contains rather uniformly distributed, fine subsolidus oxidation/exsolution lamellae. Mte.-Usp. is a 20×20-micrometer area of magnetite-ulvöspinel solid solution that appears to be homogenous. Between them is a somewhat globular baddeleyite grain. Except for the low total Cr in the ore, the deposit resembles nearby chromites in the State Line District and, indeed, is relatively near to the base of the Baltimore Mafic Complex.

Element (percent)	Ilm.-Hem.	Mte.-Usp.
O	38.18	29.05
Mg	5.49	1.26
Al	0.12	.25
Si	0.17	.31
Ti	27.05	1.44
V	.24	.77
Cr	.00	1.26
Mn	.77	.03
Fe	27.74	64.92
Ni	.00	.23
Zr	.24	.45
Total	100	100

Baddeleyite is generally thought to have a high Pb isotope closure temperature, perhaps 950 +/–100°C (J. Ramezani, pers. comm., 6/6/2004). Therefore, an attempt was made to determine when the baddeleyite was last at these higher temperatures. Six individual grains of baddeleyite from another sample of this Fe-Cr-Ti-Zr-bearing "iron ore" in serpentinite were dated by the U-Pb method Isotope Dilution-Thermal Ion Mass Spectrometry (ID-TIMS) for the present study by Jahandar Ramezani, Massachusetts Institute of Technology (pers. comm., 11/23/2003) (Table 6). They yielded slightly discordant (0.8 to 1.8 %) analyses that fit well to a discordia line with an upper intercept age of 441.7 +/- 7.3 Ma (MSWD = 0.06), the best estimate for the timing of final igneous crystallization. This appears to correspond to the Silurian Thermal and Magmatic event (STM) stage of crustal thinning and high heat flow, which also likely yielded the Arden Pluton of Aleinikoff et al. (2006). A concordia lower intercept of ~310 Ma points to an isotopic disturbance event that was likely the result of baddeleyite overgrowth, rather than lead loss. Such a large Pb loss would be rare for baddeleyite. (J. Ramezani, pers. comm., 6/2/2004). The ~310 Ma age likely corresponds to an early stage of the Alleghanian orogeny, the last stage of which was dated at 278 Ma in the Peach Bottom Slate some 12 km to the northwest (Smith and Faill 1994). Also, this ~310 Ma stage might correspond to Alleghanian duplex tectonics which advanced the BMC onto Laurentia beyond its Taconian docking position (R. T. Faill, pers. comm., 6/8/2007). SEM/EDS analyses of baddeleyite grains, most common in or on grain margins of ilmenite but also occurring in Cr-magnetite, are compared in Table 7. The differences in composition for baddeleyite associated with the two hosts suggest that baddeleyite most commonly exsolved from ilmenite to the margins of that mineral and, less frequently, from Cr-magnetite.

3) The "Model" Age of the Laurentian Continental Material: To estimate the age when detritus from the Laurentian

Table 6. U-Pb isotopic data for baddeleyite from Fulton Township, Lancaster County, Pa. All data by Jahandar Ramezani, Massachusetts Institute of Technology.

		Concentrations					Ratios						Age (Ma)[f]				
Sample Fractions	Weight (μg)[a]	U (ppm)	Pb (ppm)	Pb(c) (pg)[b]	$^{206}Pb^c/^{204}Pb$	$^{208}Pb^d/^{206}Pb$	$^{206}Pb^e/^{238}U$	err 2σ%	$^{207}Pb^e/^{235}U$	err 2σ%	$^{207}Pb^e/^{206}Pb$	err 2σ%	$^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$	Corr. Coef.[g]	Discordance (%)
b1	11.6	156	10.4	2.9	2701.7	0.041	0.069004	(.08)	0.52833	(.13)	0.05553	(.10)	430.2	430.7	433.6	0.622	0.80
b4	11.1	368	23.4	4.4	3987.7	0.018	0.068174	(.07)	0.52111	(.10)	0.05544	(.08)	425.1	425.9	429.9	0.629	1.11
b2	12.1	603	37.6	3.6	8814.0	0.003	0.068109	(.06)	0.52057	(.09)	0.05543	(.06)	424.8	425.5	429.8	0.670	1.17
b3	13.9	284	17.9	4.9	3435.0	0.004	0.067867	(.06)	0.51847	(.09)	0.05541	(.06)	423.3	424.1	428.7	0.681	1.26
b5	12.9	302	19.1	11.3	1464.5	0.004	0.066709	(.05)	0.50834	(.08)	0.05527	(.06)	416.3	417.3	423.1	0.639	1.60
b6	38.0	307	19.6	44.3	1111.5	0.002	0.066276	(.05)	0.50469	(.08)	0.05523	(.06)	413.7	414.9	421.5	0.637	1.85

[a] Sample weights are estimated by appearance using a video monitor and are known to within 40 percent.
[b] Total common Pb in analyses.
[c] Measured ratio corrected for spike and fractionation only.
[d] Radiogenic Pb.
[e] Corrected for fractionation, spike, blank, and initial common Pb.
[f] Age calculations are based on the decay constants of Steiger and Jäger (1977).
[g] "Corr. Coef." = correlation coefficient.
Mass fractionation correction of 0.15 ± 0.04%/amu (atomic mass unit) was applied to single-collector Daly analysis and 0.12 ± 0.04%/amu for dynamic Faraday-Daly analyses. Total procedural blank less than 0.6 pg for Pb and less than 0.1 pg for U.
Blank isotopic composition: $^{206}Pb/^{204}Pb$ = 19.10 ± 0.1, $^{207}Pb/^{204}Pb$ = 15.71 ± 0.1, $^{208}Pb/^{204}Pb$ = 38.65 ± 0.1.
Common-Pb corrections were calculated by using the model of Stacey and Kramers (1975) and the interpreted age of the sample.

Table 7. Compositions, in percent, of baddeleyite on contacts between ilmenite and magnetite (Grains A-C), enclosed within ilmenite (D-F), and enclosed within magnetite (G-I), Reading Iron Mine, Fulton Township, Lancaster County, 39° 43' 41.9"N, 76° 09' 50.2"W. All analyses by standardless SEM/EDS.

Oxide	On contact Grain A	On contact Grain B	On contact Grain C	In ilmenite Grain D	In ilmenite Grain E	In ilmenite Grain F	In magnetite Grain G	In magnetite Grain H	In magnetite Grain I
ZrO_2	80.84	81.97	82.03	79.66	83.32	81.74	80.21	80.01	80.37
HfO_2	1.25	1.08	1.31	2.74	1.20	1.14	1.11	1.26	1.26
TiO_2	7.51	5.97	7.09	10.39	8.27	9.91	5.24	4.05	4.85
"FeO"	10.41	10.97	9.57	7.22	7.22	7.20	13.44	13.66	13.52
Total	100.01	99.99	100.0	100.01	100.01	99.99	100.00	99.98	100.00

continental shelf, as well as the Brandywine and Baltimore massifs, was last in geochemical communication with the mantle, where it became blended with mantle material prior to formation of the island arc, a bulk composite of the refractory Fe-Cr-Ti-Zr-bearing "iron ore" was studied. This was a different sample from the same source as the six baddeleyite grains that were dated as noted above. Commercial TIMS analysis of this composite sample yielded $^{143}Nd/^{144}Nd$ = 0.513131 and, from commercial ICPMS analyses for Sm and Nd, a $^{147}Sm/^{144}Nd$ ratio of approximately 0.21357. However, these Sm and Nd concentrations were very low for the available ICPMS method, leading to a larger-than-normal uncertainty in the Sm/Nd ratio used to calculate $^{147}Sm/^{144}Nd$. From all of these data and the baddeleyite date of 441.7 +/- 7.3 Ma (J. Ramezani, Massachusetts Institute of Technology, pers. comm., 11/23/2003) K. A. Foland (Radiogenic Isotope Laboratory, Ohio State University, pers. comm., 3/18/2006) calculated an epsilon Nd of -5.7, a minimum because of uncertainties in the ICP-derived Sm/Nd elemental data. Further, he noted that such a negative epsilon Nd is inconsistent with a typical depleted mantle source (one that yielded previous melt fractions). Instead, it is consistent with either old mantle that was previously enriched with Light Rare Earth Element Enriched (LREE) crustal material (less likely) or with crustal material as expected from subduction of Laurentian detritus (more likely). Finally, Foland calculated 1.4 Ga as the maximum Nd Model age (T_{Nd}) for the crustal component. This would be consistent with the detritus having been geochemically isolated from the mantle earlier, during the Grenvillian orogeny at ~1020 Ma or the Elzeverian orogeny at ~1200 Ma. It should be noted that K. A. Foland previously (Smith 2003, Table 4) calculated T_{Nd} model ages of 1.01 to 1.04 Ga for 602.3 +/- 2 Ma igneous dikes elsewhere in eastern Pennsylvania, so the Grenvillian orogeny is a reasonable event for separation of major amounts of material from the mantle in Pennsylvania.

The date of ultimate closure of the Iapetus Ocean, during which the Goat Hill serpentinites were obducted, is as difficult to derive as the birthdate of Iapetus. Geologic processes are continuous and later events tend to obscure earlier ones. It is not unreasonable that the volcanic ash beds known as Bald Hill Bentonites A, B, and especially C, which have been identified from Virginia to New York (Smith et al. 1988 and 2003), mark such a demise. They are believed to be from island arcs in Iapetus. Other Early Paleozoic oceans are too distant to be likely sources. Work in progress by Jahandar Ramezani and Smith to provide a highly precise date for

Bald Hill Bentonite C is tending toward the boundary between the Silurian and Devonian Periods.

Table 8 is a summary of the new attempts to recognize and date events related to the creation of the serpentinites in the BMC.

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Table 8. Summary of new data used to recognize and date the development of the serpentinites of the Baltimore Mafic Complex (BMC) and surrounding area, and of recent related work by Sinha et al. (1997) and Rooney et al. (2006).

Event Interpreted or Quantified	Mineral or Rock utilized	Method of Analysis	Results	Interpretation	References and Present Cooperators
Original isolation of subducted felsic crustal component to the BMC from the mantle	Whole rock "iron ore" also enriched in CrTiZr.	Utilized TIMS data for baddeleyite and ICPMS for elemental Sm and Nd.	Maximum of 1400 Ma for T_{Nd}	Consistent with Grenvillian orogeny at ~1020 Ma and/or Elzeverian orogeny at ~1200 Ma.	Calculations by K. A. Foland, Radiogenic Isotope Laboratory, Ohio State University
Separation of ultramafic component of BMC island arc from mantle	Osmiridium	Thermal ion mass spectrometry (TIMS)	735 Ma	Consistent with T_{Nd} mantle separation of ultramafic Sword Mountain Olivine Melilitite and RRIS volcanism of Tollo and Aleinikoff (1996).	Analyses by Ryan Mathur at University of Arizona on sample collected for present study
Igneous crystallization of the BMC	Zircon	TIMS	489 +/-7 Ma	Consistent with Shaw and Wasserburg (1964)	Previous work by Sinha et al. (1997)
Taconic Orogeny in general area	Monazite	Ion microprobe	450 +/- 4 Ma	Classic date for Taconic orogeny by Wise et al. (2007). Agrees reasonably well with 453 +/- 11-Ma metamorphic growth of zircon in norite and pyroxene gabbro in BMC (Sinha et al. 1997).	Wise et al. (2007)
Stitching of obducted BMC onto Laurentia	Baddeleyite	TIMS	442 +/- 7 Ma from upper intercept	Agrees reasonably well with 0.433 Ga STM of Smith (2006).	Analyses by Jahandar Ramezani, MIT, on sample collected for present study.
Early or main phase of Alleghanian orogeny	Baddeleyite	TIMS	~310 Ma from lower intercept	Possible baddeleyite rim growth. R. T. Faill (pers. comm., 6/8/07) suspects this corresponds to Alleghanian duplex tectonics that advanced the BMC farther onto Laurentia from its Taconic docking position.	Jahandar Ramezani, as above.
Late Alleghanian lateral shear	Muscovite from mylonite zone, Peach Bottom Slate, Lancaster County	$^{40}Ar/^{40}K$ by TIMS	276 +/- 6 Ma	Mica crystallization at >300°C in fault.	Kruger Enterprises, (8/19/1994) in Faill and Smith (1994). Modern $^{40}Ar/^{39}Ar$ plateau analyses recommended.
Bald Hill Bentonite C.	Zircon	TIMS	In progress, but close to classic Silurian-Devonian boundary	From an island arc. Possibly from closure of Iapetus Ocean.	Jahandar Ramezani, as above, on samples collected for present study.

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BEDROCK COMPOSITION OF THE GOAT HILL SERPENTINE BARRENS AND A PROPOSED SERPENTINE FACTOR FOR PREDICTING FLORAL RESPONSE[1]

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ABSTRACT

The Goat Hill Serpentine Barrens tract, largely underlain by serpentinite, hosts many rare plants that thrive only on serpentinite. Because of the sequence of minerals that crystallized from the Baltimore Mafic Complex when it was still on the ocean floor, later metamorphic events after it had been obducted onto the Laurentian continental margin, and variable leaching by recent meteoric water, bedrock in the tract has a widely variable composition. Traditionally, the bedrock components MgO and Ni are believed to be positive factors for serpentine-endemic flora, and CaO and the nutrients K_2O and P_2O_5 are believed to be negative. These variables have been combined into a quantitative serpentinite factor (SF): (MgO + 50 Ni) / (10 K_2O +10 P_2O_5 + CaO). Using analyses of 35 bedrock composite samples, a plot has been generated to enable botanists to compare the distribution of flora of special concern and for others to use as one tool among many in managing the Goat Hill Serpentine Barrens in order to protect serpentine endemics and selected microenvironments and, by extension, other areas underlain by serpentinite bedrock.
[J PA Acad Sci 82(1): 31–47, 2008]

INTRODUCTION

The Goat Hill Serpentine Barrens are located in extreme southwestern Chester County, Pennsylvania. Long-term management of Goat Hill is the responsibility of the Pennsylvania Bureau of Forestry within the Department of Conservation and Natural Resources (DCNR). The nurturing of rare plant habitat is one of their primary goals for Goat Hill. Typical of many serpentinite tracts in eastern North America, Goat Hill contains a variety of unusual microenvironments that are not found elsewhere and are a consequence of its unusual bedrock chemistry, albedo, and drainage. Some serpentinite tracts are hosts to species that are holdovers from previous climates that range from the time of the retreat of the most recent glaciation from Pennsylvania, circa 18,000 years B.P., to a period of general warmth from 10,000 to 6,000 years B.P. (Webb et al., 1993). For at least the past 14,000 years there were periodic shifts to colder climates at 1650 +/– 500 year intervals (Viau et al., 2002). The last of these "little ice ages" ended circa 1850. This general warming, combined with the present trend of global warming, suggests that boreal species are in greater need of habitat protection than grasslands.

The geologic component for the Bureau of Forestry's management plan was sought from another DCNR agency, the Bureau of Topographic and Geologic Survey, which has had previous experience working in serpentinite areas. The first Pennsylvania State Geologist, H. D. Rogers, wrote "This is a range of wild and stony barrens scarcely tilled, except in a few spots on its two margins, and *overgrown with stunted black oaks, and other trees* [italics added] characteristic of the magnesian soils of all these serpentine belts" (1858, p. 170). Recently, Smith and Barnes (1998) described the geology of Nottingham County Park, 4 km to the east-northeast of Goat Hill, which is largely underlain by serpentinite having a range of compositions. The present study resulted when the Geologic Survey was asked to determine the general areas of Goat Hill that are underlain by serpentinite and potentially suitable for supporting serpentine-endemic flora. The companion paper, "Geology of the Goat Hill Serpentine Barrens, Baltimore Mafic Complex, Pennsylvania," by Smith and Barnes (2008) provides new geologic data on the area.

Although other factors, such as albedo, slope, and drainage, influence serpentine-endemic flora, Brooks (1987) surveyed the global literature and reported that there was some consensus that high levels of MgO and Ni support serpentine-endemic flora, but that they are ameliorated by CaO. Brooks considered these three elements to be the most critical components affecting serpentine-endemic flora.

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Because the usual, mineral-derived major plant nutrients K_2O and P_2O_5 support more aggressive, competing species, they are also considered herein. Though present at the level of a few thousand parts per million, the chromium in serpentinite is generally present as the extremely stable spinel-group mineral, chromite [(Mg, Fe^{2+})(Cr, Al)$_2$O$_4$], which is not available to the flora or fauna, and therefore is not included in the proposed quantified serpentine factor (SF).

Accordingly, MgO, Ni, CaO, K_2O, P_2O_5 and other elements were determined by composite bedrock sampling and analyses. Those oxides and elements thought to influence serpentine flora were combined in a quantitative serpentine factor that is herein proposed for Goat Hill and nearby serpentine tracts. Because the weighting of the individual components to calculate the quantitative serpentine factor is somewhat intuitive, raw analytical data for the individual components are included herein for possible modification of the SF equation by future researchers.

In addition to serpentine-endemic flora, serpentine barrens often have historical and cultural significance, beginning with steatite bowl manufacture by Native Americans from the southern Appalachians to Newfoundland. In Newfoundland, a spindle whorl was found that was likely reworked by Norse residents approximately 500 years before Columbus "discovered" America. Dann (1988) documented these and many other aspects of the history of serpentine.

STUDY AREA

The Goat Hill Serpentine Barrens make up an irregularly shaped tract in West Nottingham Township in extreme southwestern Chester County, Pennsylvania (Figure 1). The boundary with Maryland, the Mason and Dixon Line, forms the southern boundary of much of the tract. A meander of Octoraro Creek, forming the boundary with Lancaster County, also forms a segment of the northern boundary of the tract. Octoraro Creek drains into the Susquehanna River, which enters the Chesapeake Bay 14 kilometers southeast of that confluence. Elevations in the Goat Hill Serpentine Barrens range from approximately 175 to 350 feet above sea level and many north-facing slopes are well shaded.

Soils in the Goat Hill Serpentine Barrens are typically thin by nature, but were enhanced in thickness and nutrient burden by past agricultural and other practices. Although serpentine bedrock typically yields neutral to alkaline soils, local geochemical anomalies have also yielded acidic soils, both wet and dry. A flood plain on the southeast side of Octoraro Creek, 1 km northwest of Goat Hill proper, is well above present creek levels. It contains thick alluvium that effectively isolates the flora from serpentine bedrock. Portions well away from the bank of Octoraro Creek might be suitable for the careful disposal of non-endemic species that have been removed from flora test sites.

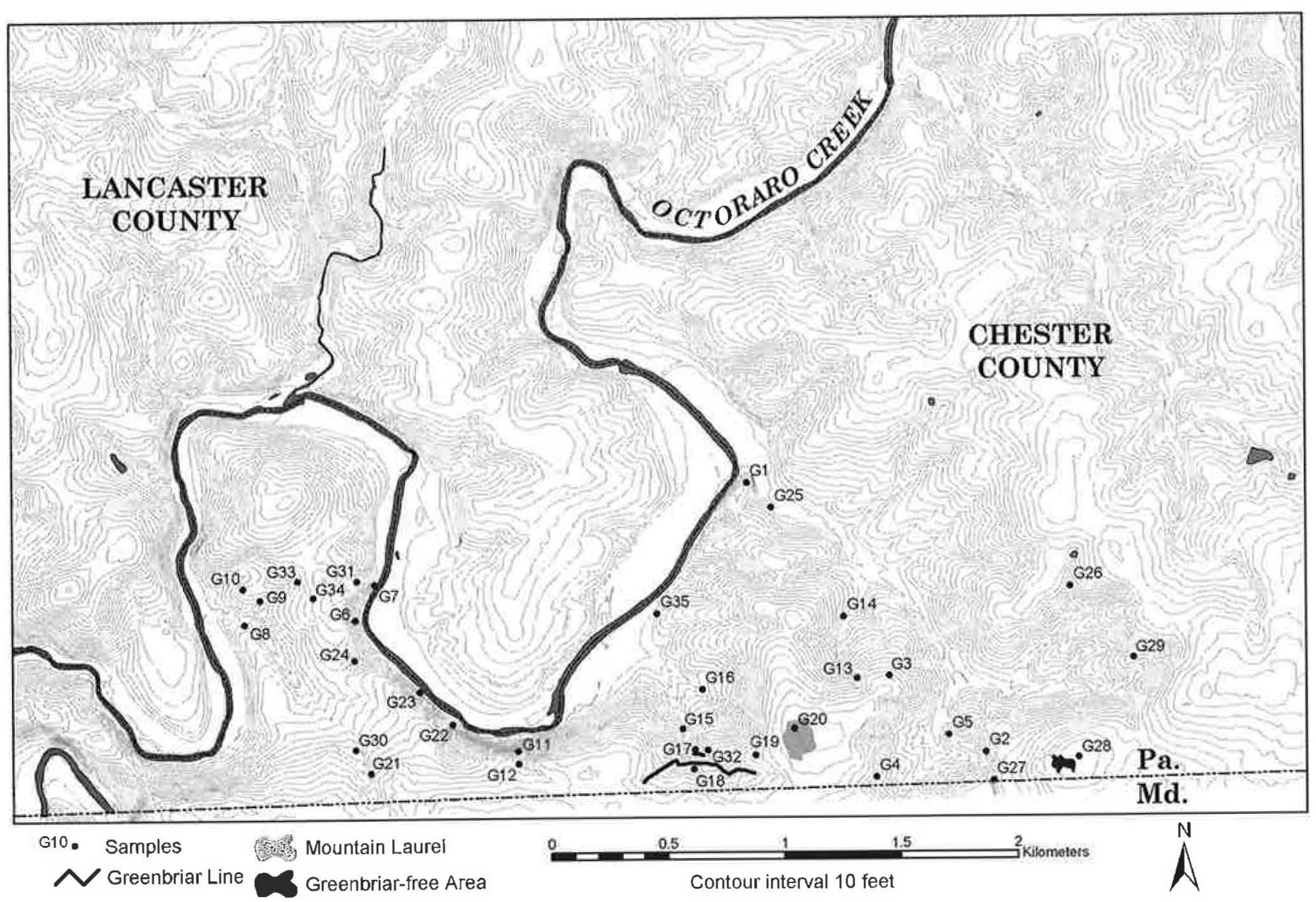

Figure 1. Location of samples collected for analysis in the Goat Hill Serpentine Barrens, Chester County, Pennsylvania. The "Greenbriar Line" marks the southern limit of luxuriant *Smilax* growth and likely represents the contact between serpentinite and overlying gabbro to the south. Management goals should not be directly related to serpentine in the "Mountain Laurel" and "Greenbriar-free" areas shown on this map, but protection is still encouraged in those areas. Pine Run, which appears to be introducing a substantial nutrient load into the Goat Hill tract, is the northwest-flowing creek that enters Octoraro Creek near sample G1.

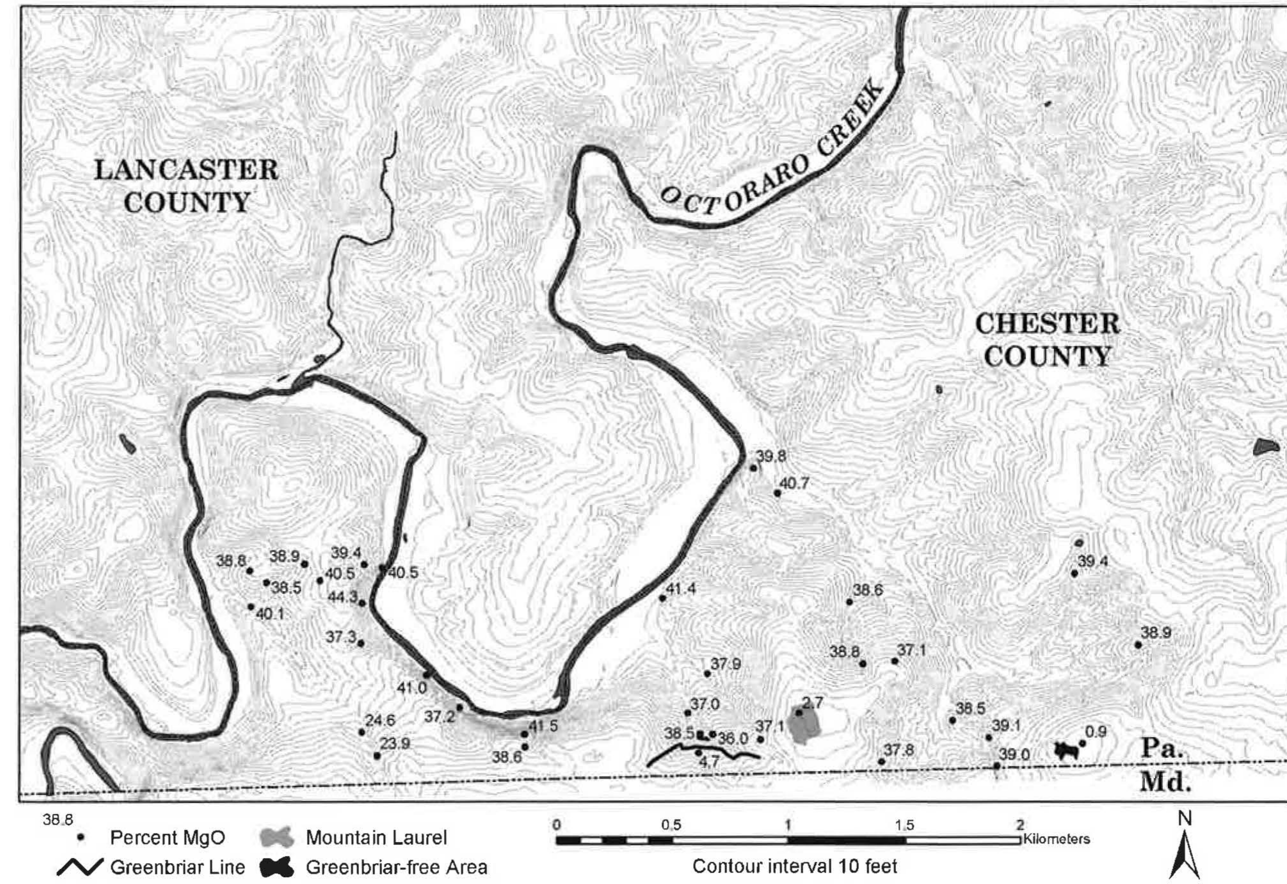

Figure 2. Plot of sample locations in the Goat Hill tract showing the concentration of MgO for each sample. For serpentine-endemic flora, it is desirable that the MgO concentration be as high as possible, preferably greater than 38 percent.

Clyde Reed (1986, p. 137) noted "The serpentine barrens of Pennsylvania have been a source of botanical interest over 100 years. William Darlington in 1853 in *Flora Cestrica* listed *Cerastium oblongifolium*, *Talinum teretifolium*, *Arabis lyrata* and *Arenaria stricta* from the barrens in Chester County. Some of his collections cited below date back to 1828. Other collectors before 1880 were William E. Cook, R. C. Alexander, M. W. Gwaddel and H. Jackson. The most extensive collector in Lancaster County was Joel J. Carter in the area about Pleasant Grove.

"From 1880 to 1900, many collectors are represented by the collections of plants from the various serpentine barrens in Pennsylvania, among them being Thomas Seal, U. C. Seal, M. E. Leeds and A. H. Leeds, Alexander MacElwee, George M. Beringer, J. B. Brinton, Ida A. Keller, William Trimble, B. H. Smith, Benjamin Heritage, John W. Eckfeldt, C. D. Lippincott, F. Windle, Charles Schäffer, and C. E. Smith. From 1900 to 1920 (and later), other collectors include Frances W. Pennell, Witmer Stone, Bayard Long, Thomas. S. Githens, E. J. Cresson, Jr., E. B. Bartram, Joseph C. Crawford, Albrecht Jahn, S. S. Van Pelt, S. C. Williamson, A. Haines, S. D. Kenney, Harold St. John and Joseph H. Painter.

"Collectors of the Pennsylvania serpentine barrens from 1920 to 1935 (or later) include J. M. Fogg, Jr., J. W. Adams, M. T. Adams, Ralph Tash, H. E. Stone, H. P. Kelley, R. R. Driesbach, R. R. Tatnall, Mary H. Williams, Walter M. Benner, William R. Taylor and H. M. Meredith. Since 1935 collectors have included E. T. Wherry, George R. Proctor, Samuel C. Palmer, L. F. A. Tanger, Hans Wilkes and Mary Domville." Reed (1986, p. 798) also notes several collections in the Goat Hill study area by Joel J. Carter, Hugh E. Stone, Bayard Long, and L. E. Anderson/R. McVaugh/G. M. Tees from 1920 to 1935.

According to Reed (1986, p. 137–138, nomenclature as reported), "Plants especially well-developed on the Pennsylvania serpentine barrens include: Adiantum pedatum var. aleuticum, Bouteloua curtipendula, Deschampsia caespitosa, Panicum annulum, Sprobolus heterolepis, Carex bicknellii, Fimbristylis baldwiniana, Scleria pauciflora var. caroliniana, Smilax herbacea subsp. crispifolia, Quercus x bushii, Polygonum tenue, Talinum teretifolium, Arenaria stricta, Cerastium arvense var. villosissimum and var. villosum, Asclepias verticillata, Phlox subulata and Aster depauperatus. Polygala verticillata was especially studied by Pennell (1931). P. pretzii Pennell and P. ambigua Mitt. were also found in the area."

For the study area itself, Reed (1986, p. 133) reports "Goat Hill, Mt. Ararat, and Ox-Bow of the Octoraro: The serpentine barrens at the southwest corner of Chester County. Many plants of interest are found along the cliffs of the Ox-Bow of Octoraro, as Campanula rotundifolia, Adiantum pedatum var. aleuticum, both indicator plants; Cerastium arvense and Arabis lyrata." His indicator plants were also observed during the present study.

Greenbrier (*Smilax*) thrives in many areas of Goat Hill where soils were artificially enhanced, making off-trail reconnaissance and sampling difficult. Because suppression of *Smilax* is one of the secondary management goals of DCNR, some observations are offered in Results and Discussion to minimize availability of major nutrients and water.

Most of the Goat Hill tract is underlain by ultramafic rocks of the Baltimore Mafic Complex (BMC). As discussed by Smith and Barnes (2008), the BMC includes portions of the ocean floor and imbedded island arcs of the former Iapetus Ocean that were obducted (thrust) up over the edge of the margin of the Laurentian continent when that ocean closed.

As described by McKague (1964) and later by Hanan and Sinha (1989), the Baltimore Mafic Complex (BMC) exposed along Octoraro Creek and at other outcrops just west of the Goat Hill tract generally comprises successively higher magmatic, cumulate layers of forsterite, orthopyroxenes, clinopyroxenes, calcic plagioclase, and mixtures thereof overlain by gabbro. The first three of these have been largely metamorphosed and/or altered to serpentine-group minerals by low-temperature reactions. The resulting serpentinite rocks superficially resemble one another despite distinct differences in MgO and Ni, which should be favorable to serpentine endemics, and CaO, K_2O, and P_2O_5, which are unfavorable.

Though it is not biologically available to the flora directly, chromite is, nevertheless, important to the present study. The presence of chromite mines and prospects, as well as slight enrichments of disseminated chromite in rock or chromite black sand concentrates in even the smallest erosion gullies, are good field indicators of forsterite formed early in the original magma chamber. These areas are potentially favorable to serpentine endemics if CaO has not been

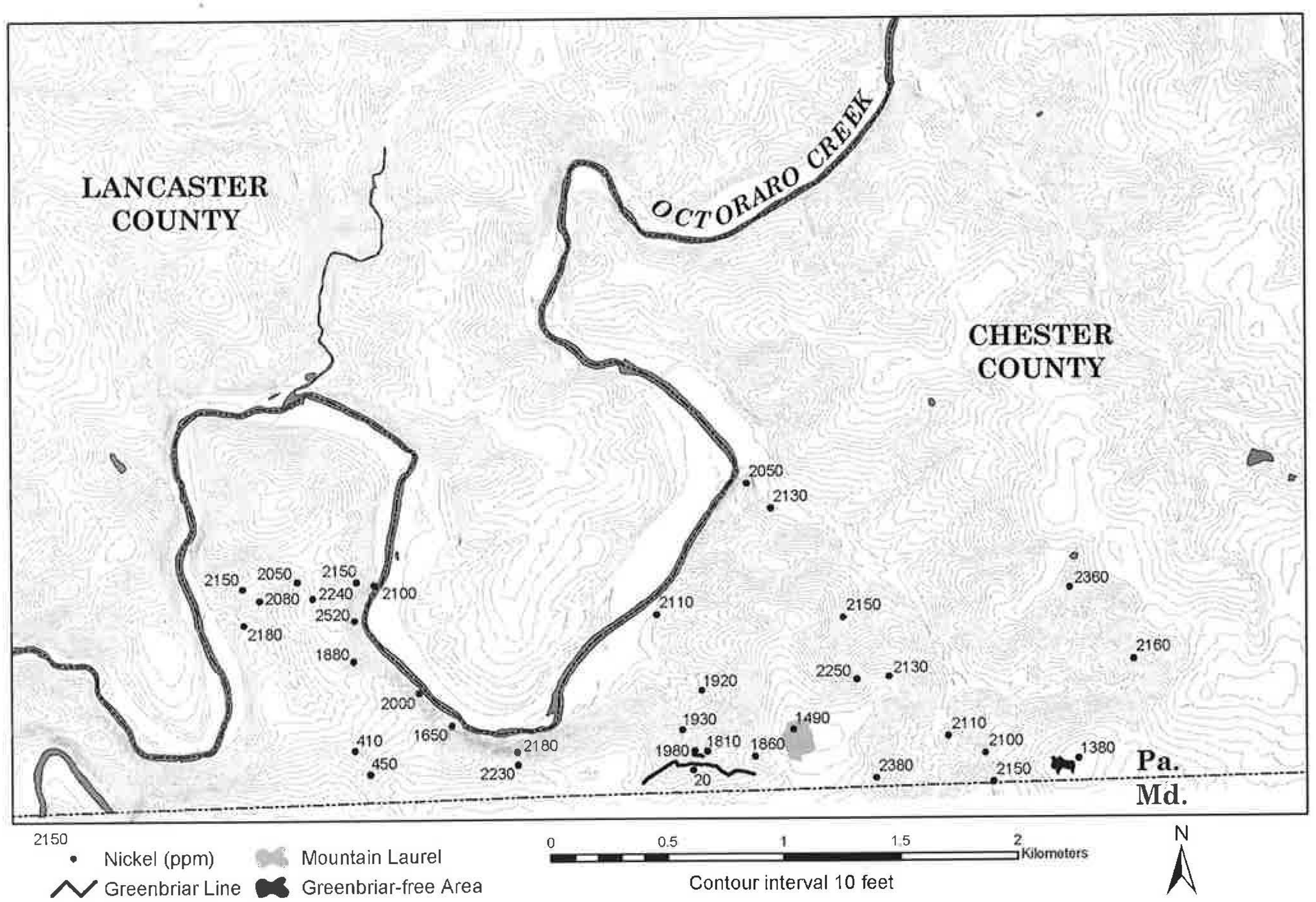

Figure 3. Plot of sample locations in the Goat Hill tract showing the concentration of Ni for each sample. For serpentine-endemic flora, it is desirable that the Ni concentration be as high as possible, preferably equal to or greater than 2100 ppm.

Table 1. Locations of Goat Hill bedrock composite samples[1]. GPS-derived sample locations have typical instrument-reported errors of 25 +/– 5 feet.

Sample Number	Latitude, N. Longitude, W in degrees, minutes, and seconds.	Comments
G1	39 43 59.5 76 05 32.2	11+/–1 m-long, prospect trench trending N12+/–2°W, located 9+/–1 m in elevation above the Peters Creek Formation.
G2	39 43 21.3 76 05 07.3	From dump to Goat Hill magnesite mine under NW high tension power line.
G3	39 43 32.2 76 05 07.3	From dump to ~5 × 3-m talc-prospect pit, 15+/–2 m SE of a N-S trail.
G4	39 43 18.2 76 05 10.0	From dump to 15+/–2-m-long trench trending S25°W, ~10 m NE of an apparent property boundary.
G5	39 43 23.8 76 04 56.9	From dumps to 2 pits, one on either side of a trail trending N35°W.
G6	39 43 42.2 76 06 44.3	From dump to Hillside Chromite Mine overlooking W side of Octoraro Creek.
G7	39 43 46.6 76 06 39.7	From intermittent outcrops over 10 m along WNW shore of Octoraro Creek, ~ ~100 m S of boundary of the Goat Hill tract.
G8	39 43 42.0 76 07 03.5	From 14+/–1-m-long outcrop on SSW bank of a tributary to Octoraro Creek flowing N80°W, ~95 m upstream of NW boundary of the tract.
G9	39 43 45.8 76 07 00.6	From dump to a 9-m-long prospect ~4 m NW of a N50°E-trending hollow in an area having 4 other prospects.
G10	39 43 47.3 76 07 04.5	From boulders and possible outcrop, ~10 m S into serpentinite from contact with Peters Creek Formation. Sampled area is 21+/–2 m S62+/–2°E of a Port Deposit Gneiss corner marker at W end of an E-W boundary segment.
G11	39 43 23.5 76 06 14.6	From an outcrop that is 10 +/–1 m long and contains a subhorizontal magnesite vein that is 0.1 to 0.2 m thick. Possibly prospected. Might yield an alkaline, high-MgO microenvironment.
G12	39 43 22.2 76 06 14.5	From outcrops to a prospect, 5 x 5 x 2 m deep, on SE side of a gumdrop hill. Center of pit appears to be ~2 m NE of S border of tract. From pit it is S71 +/–1°E to corner post across hollow. Much metagabbro float has been dumped into the head of the hollow and will diminish serpentine factor to N.
G13	39 43 32.0 76 05 13.1	Bedrock composite from 3 outcrops in a dry hollow. Uppermost outcrop is where 2 hollows join to form a N18+/–5°W trending segment. The middle outcrop is 45 +/–3 m downhill in the gully. The third is ~40+/–3 m downhill in a N38+/–3°W direction. From this third outcrop, it is 62+/–5 m down gully to Still Tender Hollow and 125+/–20 m farther down gully to a more significant gully entering from S70+/–10°W, which has acidic *Sphagnum* and *Vaccinium*.
G14	39 43 40.6 76 05 15.3	Outcrop, 7+/–1 m long, on W side of N-S tributary 250+/–25 m up gully from Pine Run. Minor acidic *Sphagnum*, *Lycopod* and *Vaccinium corymbosum* (sp.?, high bush) near outcrop, but 100+/–20m down gully from acid site mentioned above (G13).
G15	39 43 25.5 76 05 44.8	From an intermittent outcrop extending 17+/–1 m laterally and, locally, up to ~4 m high, on W side of a N-S gully 54+/–10 m down-gully from junction with a substantial gully entering from the SSW.
G16	39 43 30.9 76 05 41.1	From outcrop on SSW bank of N40°W-trending gully, where topography begins to rise toward the SE. The outcrop is 7+/–1 m long.
G17	39 43 22.6 76 05 42.6	From outcrop on SW side of a gully, 60+/–10 m upstream from the substantial gully entering from the SSW, mentioned in G15, above. This outcrop is 15+/–2 m long. From G17 SE to where the property boundary crosses the gully is 145+/–15m. G17 collected from near S edge of *Smilax* (= top of serpentinite), but Smilax on NE side of hollow is displaced to the NW relative to that on the SW side, suggesting a hollow-parallel fault.
G18	39 43 19.9 76 05 43.0	Composite of meta-anorthositic gabbro float from *Fagus-Quercus* forest lacking conifers and 23+/–2 m south of the *Smilax*. Sample from 47+/–3 m S53+/–2°W of a Port Deposit Gneiss corner boundary. Gabbro possibly slumped from Maryland, but trees not seeing serpentinite.
G19	39 43 21.6 76 05 31.7	Composite of mined talc-magnesite-chlorite steatite from W pit of two pits that are 37+/–3 m apart and along the S boundary. The sampled pit is ~159+/–15 m W of the more southerly of two Port Deposit Gneiss corner markers that are 10.5+/–1 m apart on the N-S boundary jog.
G20	39 43 25.2 76 05 24.6	Composite of "scoraceous," siliceous, replacement-insoluble residue of serpentinite from nearly flat Goat Hill plateau, 110+/–10 m N15+/–5°E of the more northerly of the two Port Deposit Gneiss corner markers mentioned in G19. From an area of *Kalmia latifolia* (Mountain Laurel), *Quercus*, *Vaccinium* (*pallidum* sp.?, low-bush), and some conifers, but almost no *Smilax*. Tree blow-downs reveal red hematitic soil that might have been of interest to Native Americans. Closed, shallow depressions at 39 43 22.5 N, 76 05 22.2 W and 39 43 22.0 N, 76 05 19.9 W appear to be old.
G21	39 43 20.4 76 06 41.2	Composite of talc-anthophyllite float from E side of moderate N-S gully having acidic flora, such as *Kalmia latifolia*, only on the E side. No conifers on either side, but elevation higher on E side, suggesting more resistant bedrock and a N-S fault.

(*Table 1. continued*)

Table 1. continued.

Sample Number	Latitude, N. Longitude, W in degrees, minutes, and seconds.	Comments
G22	39 43 28.2 76 06 26.2	Composite of apparently mined, fresh rock from a dump, 4.5+/–1 m long and 2.8+/–0.5 m high, 50+/–5 m NW of alluvial fan from "Deep Hollow," 23+/–2m SW of SW bank of Octoraro Creek.
G23	39 43 31.5 76 06 32.0	Composite of pyroxenite from outcrop 16 to 23 m above level of Octoraro Creek. Serpentinite at top of outcrop not included. Ca appears to be available in seeps, based on *Adiantum aleuticum* (Aleutian Maidenhair Fern), *Aquilegia canadensis* (sp.?), and *Camptosorus rhiz.* (Walking Fern). Pyroxenite does not seem to support Smilax despite availability of water.
G24	39 43 37.1 76 06 43.7	Composite of 4 pieces of mostly sheared serpentinite from each of 3 outcrops in a gully. Each outcrop is 35+/–5 m apart, zigzagging in the gully. Farthest uphill sample is 63+/–8 m down gully from abandoned township road.
G25	39 43 56.8 76 05 27.5	Composite of central 15 m from large outcrop on SW side of Piney Run at a point 125+/–10 m SE of the serpentine-gneiss contact near the confluence with Octoraro Creek.
G26	39 43 44.0 76 04 34.3	Composite from 36 to 46 m down gully from very old bridge in northeast portion of Goat Hill tract. Probably near boundary with The Nature Conservancy tract, but old and new signs are in conflict.
G27	39 43 17.8 76 04 49.0	Composite over 11 m of outcrop from 0 to 2 m above a bench-trail on E side of hollow immediately north of the Mason and Dixon line. Minor quarrying nearby.
G28	39 43 20.2 76 04 33.4	Composite of 12 pieces of Fe-Si-leached serpentinite float from near gentle crest of 450+/–15-foot hill. From sample area to corner marker is 18+/–3 m N40°E. Site is 16+/–3 m W of stone fence.
G29	39 43 33.9 76 04 23.7	Composite from 1-m-high bank to ESE of bulldozed area. This area is 8+/–2 m N65+/–5°W from E boundary in this area and 12+/–2 m S12+/–2°E to a rebar survey stake. Property boundary in this area seems to run ~N35°E.
G30	39 43 23.7 76 06 43.8	Composite of talc-anthophyllite float from 125+/–25 m N45°W of G21. This is lower in elevation and across the hollow to the NW of G21 and ~45+/–10 m W of the N-S gully. The gully appears to be fault-controlled and has more-acid flora on the higher, E side.
G31	39 43 47.1 76 06 42.9	Composite from outcrops at "Point of Rocks," 53+/–3 m S of N boundary. Little Smilax and no conifers to N, so probably at sheared base of Baltimore Mafic Complex.
G32	39 43 22.5 76 05 40.3	Composite over ~15 m outcrop having shears trending ~N80+/–5°W, N65+/–5°N. The outcrop is located 22+/–2m S of *Smilax* line. From sample to corner marker is S43+/–2°W. From G32 to G17 is 60+/–5 m down gradient or N47+/3°W.
G33	39 43 47.3 76 06 53.7	Composite of brecciated rock from a dump that is 5 x 9 m and up to 2.6 m high. The rock was mined from a water-filled shaft, 3 × 5 m, located 23+/–2 m S of N boundary of Goat Hill tract. Possibly from base Baltimore Mafic Complex. Separate chromite composite collected.
G34	39 43 45.1 76 06 50.6 39 43 44.8 76 06 51.2	Composite from dumps to twin, water-filled chromite shafts. SSW shaft 3.5 x 8 m at present water levels and NNE shaft 4 x 6 m. Center-to-center, shafts are 19+/–2 m apart on a bearing of S85+/–5°W. Area is 40+/–5 m W of abandoned township road at a point 35+/–5 m N of crest. General area of reported U.S. Bureau of Mines trenching (McIntosh and McHenry, 1948).
G35	39 43 41.6 76 05 49.0	Composite from serpentinite outcrop just above floodplain of Octoraro Creek. Serpentinite contains possible relict parting from pyroxene.

[1]Sampling dates: November 14–16, 2000; March 1–2, 12, 14–15, November 15–16, 19–20, 2001; February 28, March 1, 12–13, 15–16, 2002; June 16–17, 2003.

added by later processes. Sources of CaO include hydrothermal introduction as dolomite during metamorphism; liming by farmers in the period circa 1830 to 1930; or use of limestone or dolomite in roads, trails, and concrete. Areas of serpentine lacking significant disseminated chromite may have been derived from clinopyroxene layers formed higher and later in the magma chamber than forsterite. Bedrock sampling and analyses of such serpentines will also reveal higher, unfavorable amounts of CaO. However as will be shown, in the senior author's semi-Pennsylvania Dutch, the Goat Hill Serpentine Barrens are "wonderfully low" in CaO.

Genth (1875) reported that mines in Goat Hill produced 2,250 tons of magnesite ($MgCO_3$) in the period 1836 to 1850 and 8,500 tons between 1854 and 1871. Outcrops and rock dumps near composite samples G2 and G11 (Figure 2) might provide unusual microenvironments having high pH and readily available Mg. Acid rains might also contribute the sulfate anion for small buildups of epsomite, $MgSO_4{\cdot}H_2O$, in underhangs during the summer months.

Brucite, $Mg(OH)_2$, which would yield extremely high natural pH values, was sought but not observed during the present study. As naturally occurring brucite was common at the Wood Mine to the north and occurs in lesser amounts at the Cedar Hill and Penn/MD quarries to the west in Lancaster County, it is possible that seeps derived from deep, brucite-bearing rock might be found and yield still more extreme microenvironments. These would be most alkaline during droughts as brucite has already been leached from rock that has been above the water table for any significant period.

Other mineral prospecting sites in and adjacent to the Goat Hill tract appear to have been dug in search of com-

Table 2. Major, minor, and selected trace-element content of bedrock composites from the Goat Hill Serpentine Barrens area. Analyses by XRAL Laboratories, Ltd. Oxides and LOI reported in percent, Au, Pd, and Pt in ppb. All others reported in ppm. All have Be <0.8, Cd <1, Br ≤ 2, and Ta ≤ 3. Sample G18 of gabbro and samples G21 and G30 from higher metamorphosed igneous cumulates than the others.

SAMPLE NAME	SiO_2	Al_2O_3	CaO	MgO	Na_2O	K_2O	Fe_2O_3	MnO	TiO_2	P_2O_5	LOI
G1	37.1	0.48	0.37	39.8	<0.01	0.03	10.2	0.11	0.02	0.02	11.1
G2	39.4	0.20	0.28	39.1	<0.01	0.03	7.41	0.07	0.01	0.01	13.4
G3	40.9	0.59	0.06	37.1	<0.01	0.05	9.07	0.10	0.02	0.02	11.8
G4	39.0	0.20	0.40	37.8	<0.01	0.04	7.92	0.07	0.01	0.01	13.5
G5	40.5	0.12	0.32	38.5	<0.01	0.03	7.59	0.08	0.01	0.01	13.0
G6	35.4	0.17	0.12	44.3	<0.01	0.03	7.34	0.11	0.01	0.01	12.4
G7	37.4	0.16	0.09	40.5	<0.01	0.03	7.06	0.09	0.01	0.02	14.6
G8	39.6	0.23	0.08	40.1	<0.01	0.02	7.35	0.07	0.01	0.01	12.4
G9	41.2	0.85	0.06	38.5	<0.01	0.03	7.00	0.12	0.02	0.01	12.3
G10	38.4	0.53	0.07	38.8	<0.01	0.03	6.99	0.10	0.02	0.02	15.0
G11	36.8	0.25	0.08	41.5	<0.01	0.02	7.93	0.09	0.01	0.01	13.1
G12	39.5	0.22	1.1	38.6	<0.01	0.03	7.69	0.09	0.01	0.02	12.4
G13	39.8	0.27	0.25	38.8	<0.01	0.02	8.07	0.07	0.05	<0.01	12.4
G14	40.4	0.86	0.18	38.6	<0.01	0.02	8.20	0.11	0.07	<0.01	11.8
G15	40.1	0.22	1.0	37.0	<0.01	0.04	8.17	0.10	0.04	<0.01	12.7
G16	40.2	0.34	0.08	37.9	<0.01	0.03	7.55	0.11	0.04	<0.01	13.3
G17	40.4	0.29	0.33	38.5	<0.01	0.02	8.28	0.19	0.04	<0.01	12.1
G18	42.4	22.8	12.2	4.7	1.42	0.20	11.0	0.17	1.54	0.06	3.0
G19	36.1	0.33	0.10	37.1	<0.01	0.02	7.44	0.12	0.04	<0.01	18.6
G20	85.5	0.31	0.02	2.7	0.05	0.04	8.33	0.08	0.05	0.02	2.0
G21	53.6	2.65	0.18	23.9	<0.01	0.04	13.5	0.19	0.18	<0.01	4.6
G22	43.0	1.24	0.09	37.2	<0.01	0.03	7.00	0.09	0.06	<0.01	11.3
G23	39.8	0.23	0.01	41.0	<0.01	0.02	7.82	0.10	0.04	<0.01	11.3
G24	43.3	0.72	0.24	37.3	<0.01	0.03	6.72	0.09	0.05	<0.01	11.5
G25	37.9	0.27	0.02	40.7	0.03	0.03	7.40	0.10	0.02	<0.01	13.6
G26	38.6	0.36	0.74	39.4	0.03	0.04	7.78	0.14	0.02	<0.01	13.1
G27	39.8	0.25	0.65	39.0	0.04	0.02	7.37	0.07	0.03	<0.01	13.2
G28	90.1	0.45	0.005	0.9	0.08	0.04	7.01	0.08	0.02	0.01	1.4
G29	39.6	0.21	0.005	38.9	0.04	0.03	7.52	0.09	0.02	<0.01	13.6
G30	51.3	2.73	1.34	24.6	0.08	0.03	14.7	0.29	0.23	<0.01	4.8
G31	37.7	0.13	0.06	39.4	0.03	0.02	6.47	0.08	0.01	<0.01	16.5
G32	33.6	0.31	0.10	36.0	0.06	0.03	6.66	0.13	0.02	<0.01	23.5
G33	39.2	1.01	0.09	38.8	0.03	0.02	7.39	0.12	0.04	<0.01	12.4
G34	40.4	0.74	0.04	40.5	0.03	0.03	5.95	0.07	0.02	<0.01	11.9
G35	37.5	0.21	0.005	41.4	0.02	0.03	7.32	0.10	0.02	<0.01	13.4

(*Table 2. continued*)

mercial deposits of albite, chromite, quartz, and talc. Prehistoric mining for steatite, chert, and red pigment cannot be ruled out.

METHODS

In order to estimate the serpentine factor for various portions of the Goat Hill tract, it was necessary to collect and analyze composite bedrock samples throughout the tract. Outcrops are scarce in the Goat Hill tract. Accordingly, natural ledges in stream gullies and dumps to abandoned mines and prospects were targeted. For the latter, an attempt was made to avoid mineralized rock. At each potential sampling site, 15 fist-size samples, believed to represent bedrock at that site, were collected, initially as larger pieces, at roughly equal distances across the available outcrop. After they were laid out in order on a canvas sack, samples were trimmed to fist-size. In a few cases, additional material was collected to bring smallish sub-samples up to size. After field trimming and sizing, composites were made of the 12 freshest cores that seemed to represent normal bedrock at that site. The other 3 were discarded. The locations of the Goat Hill bedrock composites are presented in Table 1 and shown in Figure 1.

In the rock preparation laboratory, each sample was crushed to −1 cm using a TM Engineering Ltd. jaw crusher. Then an approximately 200-ml split was stored in a Nalgene bottle for archival purposes and a separate 60-g split was ground to −100 mesh using a Rocklabs ring mill and chrome head.

Commercial analyses were obtained for major and minor elements by X-ray fluorescence and trace elements by inductively coupled plasma and instrumental neutron activation analysis. Major, minor, and relevant trace element analyses are presented in Table 2. X-ray powder diffraction (X.R.D.) analyses to determine the major and minor minerals in each serpentinite sample were done in-house.

Table 2. Continued

SAMPLE NAME	Ag	As	Au	B	Ba	Co	Cr	Cs	Cu
G1	<0.2	25	<5	30	61	129	4710	2	3.9
G2	<0.2	3	6	34	83	98	2550	2	1.6
G3	0.3	2	<5	31	65	108	2610	<1	3.2
G4	<0.2	2	<5	29	74	110	3870	<1	0.7
G5	<0.2	5	<5	<10	67	100	2330	3	0.9
G6	0.3	3	<5	12	61	103	2810	2	2.3
G7	<0.2	56	17	110	77	99	2310	1	2.5
G8	<0.2	50	8	19	71	98	2470	2	2.1
G9	<0.2	9	<5	<10	80	92	2430	<1	2.0
G10	0.2	6	<5	<10	50	90	3490	2	3.5
G11	<0.2	2	<5	35	79	106	2440	2	1.7
G12	<0.2	39	<5	41	70	99	2830	2	5.0
G13	<0.2	211	29	21	10	89	2610	<1	4.0
G14	0.2	111	7	37	10	90	2690	1	5.6
G15	<0.2	2	<5	26	30	78	2280	1	2.4
G16	<0.2	0.5	<5	20	21	75	2370	<1	3.0
G17	<0.2	46	<5	38	31	80	2890	1	3.6
G18	<0.2	0.5	<5	30	35	28	127	3	44.6
G19	0.2	69	<5	23	10	77	2220	1	5.9
G20	<0.2	65	<5	25	40	109	3070	2	3.9
G21	<0.2	10	36	30	32	71	1640	2	280
G22	0.4	3	6	27	10	70	1910	2	4.3
G23	0.3	20	<5	30	10	81	2380	1	3.8
G24	<0.2	5	<5	22	10	74	2210	2	4.2
G25	0.2	16	<5	43	31	103	2730	<1	1.3
G26	<0.2	5	<5	47	59	127	2540	<1	3.0
G27	<0.2	41	6	28	38	97	2560	<1	6.8
G28	<0.2	4	5	13	66	156	3310	1	6.1
G29	<0.2	2	<5	31	38	104	2650	<1	1.2
G30	<0.2	24	5	42	72	84	1430	2	15.7
G31	<0.2	4	<5	20	48	99	2320	<1	1.4
G32	<0.2	48	<5	17	53	89	2150	<1	11.9
G33	<0.2	83	<5	44	34	89	5000	<1	3.2
G34	<0.2	20	<5	31	28	84	6480	1	1.9
G35	<0.2	0.5	<5	60	44	103	2770	2	1.0

(Table 2. continued)

RESULTS AND DISCUSSION

Anthropogenic impacts

The present distribution of flora likely reflects a substantial anthropogenic impact that began with the introduction of European agricultural methods and invasive species. No evidence of floral modification by Native Americans using fire exists in this area (Kurt Carr and Robert Winters, personal communications, 2000). Because of the shallowness and low fertility of natural, serpentine-derived soils, these areas were likely to be some of the last to be farmed and the first to be abandoned in southeastern Pennsylvania. Also, farmers in the period circa 1830 to 1930 applied lime to the fields to improve fertility (Francis Kirk, personal communication 1978). Likely, they also applied organic fertilizers intentionally or through animal grazing. The name Goat Hill itself suggests grazing. The effects of organic fertilizer might be discernable through comparison of relative tree-ring thicknesses with drought records and by analyses of low-temperature ash composites. (For this and other reasons, the oldest surviving trees in the tract should be identified and protected.) Many remaining fragments of pastures visible on older aerial photographs are rimmed by lush growths of greenbrier downslope. These rims might be gradually ameliorated by cutting brush, which presently acts as a reservoir for nutrients and prevents solar baking, and removing it as wood chips. These chips should then be hauled off areas underlain by serpentine bedrock before they have a chance to release nutrients through decay or combustion.

Based on algal growth in summer, Pine Run (Figure 1) appears be introducing a substantial nutrient load into the Goat Hill tract. Some of this might be bioharvested through judicious removal of dead and mature trees where the tributaries enter the tract. Replacement plantings of rapid growth, native species, or even Sphagnum, might be considered. Deer are also likely vectors bringing nutrients into the tract. Enhanced deer harvesting might be encouraged. Rabbits, on the other hand, are probably not as wide ranging and tend to girdle *Smilax* during snow cover. Reduction of rabbit predators such as fox might receive support from pheasant-friendly groups.

Table 2. Continued.

SAMPLE NAME	Ni	Pb	Pd	Pt	Rb	Sb	Sc	Se	Sn	Sr	Th	U
G1	2050	5	18	12	4	0.8	4	<3	3	8	1.0	1.2
G2	2100	4	10	<10	2	<0.2	5	<3	4	7	0.8	1.6
G3	2130	6	<1	<10	3	0.5	6	5	3	6	<0.5	<0.5
G4	2380	5	<1	<10	2	0.4	5	<3	4	7	1.2	1.2
G5	2110	4	16	<10	2	0.3	6	7	3	6	0.6	0.5
G6	2520	5	12	<10	5	0.5	4	5	4	5	<0.5	0.6
G7	2100	3	<1	<10	5	1.5	6	<3	4	6	<0.5	0.6
G8	2180	3	12	<10	2	0.8	6	<3	3	4	1.5	1.1
G9	2080	8	<1	<10	5	0.8	6	<3	4	6	0.5	2.6
G10	2150	4	<1	<10	4	0.8	6	6	3	6	1.0	1.3
G11	2180	4	14	<10	3	0.7	7	<3	3	7	1.2	0.5
G12	2230	4	11	<10	4	1.4	6	6	4	15	<0.5	<0.5
G13	2250	<2	12	<10	6	9.0	4	<3	3	5	0.6	1.3
G14	2150	<2	<1	<10	5	6.7	5	<3	4	3	<0.5	1.1
G15	1930	<2	17	<10	7	0.2	5	<3	4	7	1.0	<0.5
G16	1920	<2	11	<10	5	0.3	5	<3	4	3	0.5	<0.5
G17	1980	<2	<1	<10	7	0.5	5	<3	3	5	0.7	1.0
G18	24	<2	17	<10	8	0.3	28	<3	3	284	<0.5	2.5
G19	1860	2	14	<10	7	1.9	5	<3	5	2	1.3	1.9
G20	1490	3	15	<10	12	1.6	3	5	4	5	0.9	0.8
G21	454	2	54	162	9	<0.2	45	<3	3	2	1.2	1.9
G22	1650	<2	11	<10	7	0.8	5	3	4	4	1.1	2.1
G23	2000	<2	<1	<10	4	0.8	5	<3	4	<2	0.6	0.5
G24	1880	<2	12	<10	7	0.3	5	6	3	5	<0.5	1.3
G25	2130	4	<1	<10	7	1.5	5	<3	4	7	1.0	0.9
G26	2360	3	<1	<10	4	0.6	4	<3	5	16	<0.5	<0.5
G27	2150	3	<1	<10	7	<0.2	4	<3	3	17	<0.5	<0.5
G28	1380	4	<1	<10	15	<0.2	4	<3	4	6	<0.5	<0.5
G29	2160	2	<1	<10	4	<0.2	4	<3	3	8	<0.5	0.9
G30	409	3	24	45	8	<0.2	45	<3	3	10	<0.5	<0.5
G31	2150	<2	<1	<10	8	<0.2	4	<3	4	9	<0.5	1.0
G32	1810	<2	19	<10	6	0.6	4	<3	4	8	0.6	1.1
G33	2050	2	<1	<10	5	2.6	4	4	2	9	<0.5	0.6
G34	2240	3	<1	<10	7	0.6	3	<3	3	8	0.7	1.3
G35	2110	2	12	<10	5	0.7	5	<3	3	5	<0.5	<0.5

(Table 2. continued)

Serpentine bedrock in the tract typically contains less than 100 ppm S, the lower detection limit for this study. Thus, prior to increases in atmospheric SO_2 from coal-burning power plants, especially in the Midwest, S may have been a limiting nutrient for flora in the tract. This might be verified through sulfur isotope analyses of low-temperature ash composites of dated tree rings. Although not determined during the present study, serpentine bedrock is likely to have a delta S^{34} value near zero, whereas SO_2 from combustion of high-sulfur coals is likely to be much higher. If such a study verifies the positive effect of anthropogenic sulfur on tree growth in the tract, judicious timber harvesting to remove S as well as potash, phosphorus, and nitrogen should assist with creation of grassland in geologically favorable areas. Certainly, it is widely accepted that, elsewhere in Pennsylvania, such S has promoted growth of deciduous trees on quartzite and sandstone uplands that are typically deficient in S.

Smilax *Suppression*

Rampant *Smilax* growth appears to be a good negative measure of the health of the barrens and is deleterious in itself. Although it would be extremely difficult to eradicate *Smilax*, and perhaps even undesirable for some species, suppression might be achieved by managing sunlight, water and nutrients. First, it should be mentioned that suppression is likely achievable in the Goat Hill tract. Unlike the situation to the east at Nottingham County Park, the bedrock underlying Goat Hill is lower in natural levels of calcium, there are fewer areas that retain water in the drainage gullies, and the nutrients stored in the forest canopy and brush are not as rapidly recycled or made more readily available by fire. Thus, *Smilax* at Goat Hill is less luxuriant than at Nottingham County Park and the former tract has a potentially higher value for plant restoration.

As noted above, removal of timber and brush from the rims of grasslands should decrease the nutrient burden, transmit more sunlight to dark serpentine to enhance baking,

Table 2. Continued.

SAMPLE NAME	V	Y	Zn	Zr	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
G1	<10	3	26	28	<0.5	12	12	<0.1	<0.2	<0.5	0.3	0.06
G2	<10	3	27	38	<0.5	14	9	0.1	<0.2	0.6	0.3	0.12
G3	<10	4	33	28	0.6	6	11	<0.1	<0.2	<0.5	0.5	0.14
G4	<10	3	31	28	<0.5	<3	8	<0.1	<0.2	<0.5	<0.2	0.1
G5	<10	<2	24	22	<0.5	15	12	<0.1	<0.2	0.5	0.2	0.05
G6	<10	3	25	32	<0.5	7	<5	<0.1	0.2	<0.5	0.3	0.06
G7	<10	2	23	27	<0.5	12	<5	<0.1	<0.2	<0.5	0.4	0.1
G8	<10	2	24	24	<0.5	18	10	<0.1	<0.2	<0.5	0.2	0.1
G9	12	3	35	33	1.0	8	7	0.1	<0.2	<0.5	0.4	<0.05
G10	<10	<2	24	32	<0.5	4	7	0.1	<0.2	<0.5	0.5	0.21
G11	10	3	27	33	<0.5	18	9	<0.1	<0.2	0.5	<0.2	0.07
G12	<10	<2	24	22	<0.5	8	6	<0.1	<0.2	<0.5	0.4	0.12
G13	<10	4	32	31	0.6	5	5	<0.1	0.2	<0.5	0.5	0.07
G14	19	5	35	27	0.9	<3	<5	<0.1	0.2	<0.5	0.4	0.05
G15	<10	3	33	23	<0.5	<3	11	<0.1	<0.2	<0.5	<0.2	<0.05
G16	<10	4	26	25	<0.5	<3	9	<0.1	<0.2	<0.5	<0.2	0.08
G17	11	5	23	26	<0.5	<3	8	<0.1	0.2	<0.5	<0.2	0.07
G18	206	8	83	38	1.8	3	<5	0.4	0.7	<0.5	0.6	0.05
G19	13	5	25	29	<0.5	5	10	<0.1	<0.2	<0.5	0.3	0.07
G20	11	<2	37	11	5.3	16	10	1.7	0.3	<0.5	1.1	0.15
G21	143	7	85	19	2.1	<3	7	0.3	0.3	0.6	<0.2	<0.05
G22	18	4	33	29	<0.5	<3	6	0.1	<0.2	<0.5	0.4	0.07
G23	<10	6	29	25	<0.5	4	<5	<0.1	<0.2	<0.5	<0.2	0.06
G24	17	5	39	28	<0.5	10	<5	<0.1	0.3	<0.5	<0.2	<0.05
G25	<10	3	20	25	<0.5	7	9	<0.1	0.3	1.5	<0.2	0.06
G26	<10	4	23	22	<0.5	4	<5	<0.1	0.2	<0.5	0.2	0.05
G27	<10	<2	25	30	<0.5	<3	6	<0.1	0.3	<0.5	<0.2	0.07
G28	<10	<2	44	8	1.4	19	<5	0.6	0.2	<0.5	0.2	0.1
G29	<10	5	21	28	<0.5	3	8	<0.1	0.3	<0.5	<0.2	<0.05
G30	172	5	103	16	0.8	4	5	0.3	0.3	0.6	<0.2	<0.05
G31	<10	3	171	27	<0.5	7	7	<0.1	0.2	<0.5	<0.2	<0.05
G32	<10	<2	16	27	<0.5	13	<5	<0.1	<0.2	1.2	<0.2	<0.05
G33	<10	5	34	36	<0.5	20	11	0.1	0.2	<0.5	0.2	0.06
G34	<10	5	15	27	<0.5	<3	7	<0.1	<0.2	<0.5	<0.2	<0.05
G35	<10	4	20	24	<0.5	<3	<5	<0.1	<0.2	0.8	<0.2	0.07

and reduce water. Such brush clearing should be especially effective on south-facing slopes and ridge tops. Water on such ridge tops in particular might be decreased further by excavating free-draining trenches perpendicular to ridge crests. This may appear counterintuitive to soil conservationists, but many serpentine endemics seem to do best on nearly barren rock. Likewise, once the nutrient and fuel load has been greatly reduced through cutting and hauling off site, artificial, low-intensity controlled burns might be useful to help maintain grasslands if all reasonable safety issues can confidently be addressed. Fire-stained rock (ferrous iron oxidized to red ferric iron) was not observed during the present study. Absence of evidence of burns by Native Americans is consistent with present understanding of their cultural practices in southeastern Pennsylvania. (Such general absence might be confirmed with fission-track dates of the mineral apatite, which typically resets at about 100°C.) As an additional management strategy, it would likely be beneficial to remove accumulated organic material where there is serpentine bedrock of appropriate composition at shallow depth. Areas on the upper floodplain of Octoraro Creek northwest of Goat Hill might be suitable for carefully bioengineered disposal. Avoidance of N-P-K rich runoff is especially desirable because of the hydrologic connection to the Chesapeake Bay, whether runoff is derived from slow decay of organic matter or more rapidly from burns on serpentine bedrock noted for its high surface runoff.

Other habitats and possible microenvironments

In addition to the grassland savannah areas which conceivably might reflect much migrated relicts of a drier climate 10,000 to 6,000 years B.P., Goat Hill also has some treasured cool, moist, and shaded habitat along the north side of the hill facing Octoraro Creek. Unlike the grasslands, some of these tolerate or even require a well-developed tree canopy. One such area observed during the present study supports the Aleutian, boreal form of the maiden hair fern (*Adiantum aleuticum*), wild columbine (probably *Aquilegia canadensis*, but dwarfed or stunted), and walking fern (*Asplenium rhizophyllum*). Just as at the Tablelands serpentine barrens at Gros Morne National Park, Newfoundland,

calcium is supplied by local seeps. This environment also serves as a reminder that Pennsylvania has survivors of the boreal climate which, except for brief 1650 +/– 500 year cold cycles, ended in Pennsylvania 13,000 years B.P. Regardless of its cause, global warming is in progress, obviously making these serpentine-dependent habitats of boreal species in greater need of management than grasslands, which can flourish on many rock and soil types.

Areas where an increase in sunlight might be beneficial include those where glade spurge (*Euphorbia purpurea*) survives. Several nearby sectors along the southwest side of Pine Run support an extremely acid-loving flora, unlike the "normal" situation in serpentine barrens. Such acid-rich areas result from dissolution of Fe^{2+}-bearing olivine currently undergoing natural dissolution in the subsurface of Goat Hill. This dissolution reaction can be expressed schematically as follows:

$$Fe^{2+} \text{ (from olivine)} + 3HOH \text{ (water)} = \text{"}Fe(OH)_3\text{" (yellow boy)} + 3H^{+} \text{ (acid)}$$

(This reaction requires some oxygen and does not yield net free acid. However, in some situations, ferrous iron dissolved from forsterite, a type of olivine, can be separated by

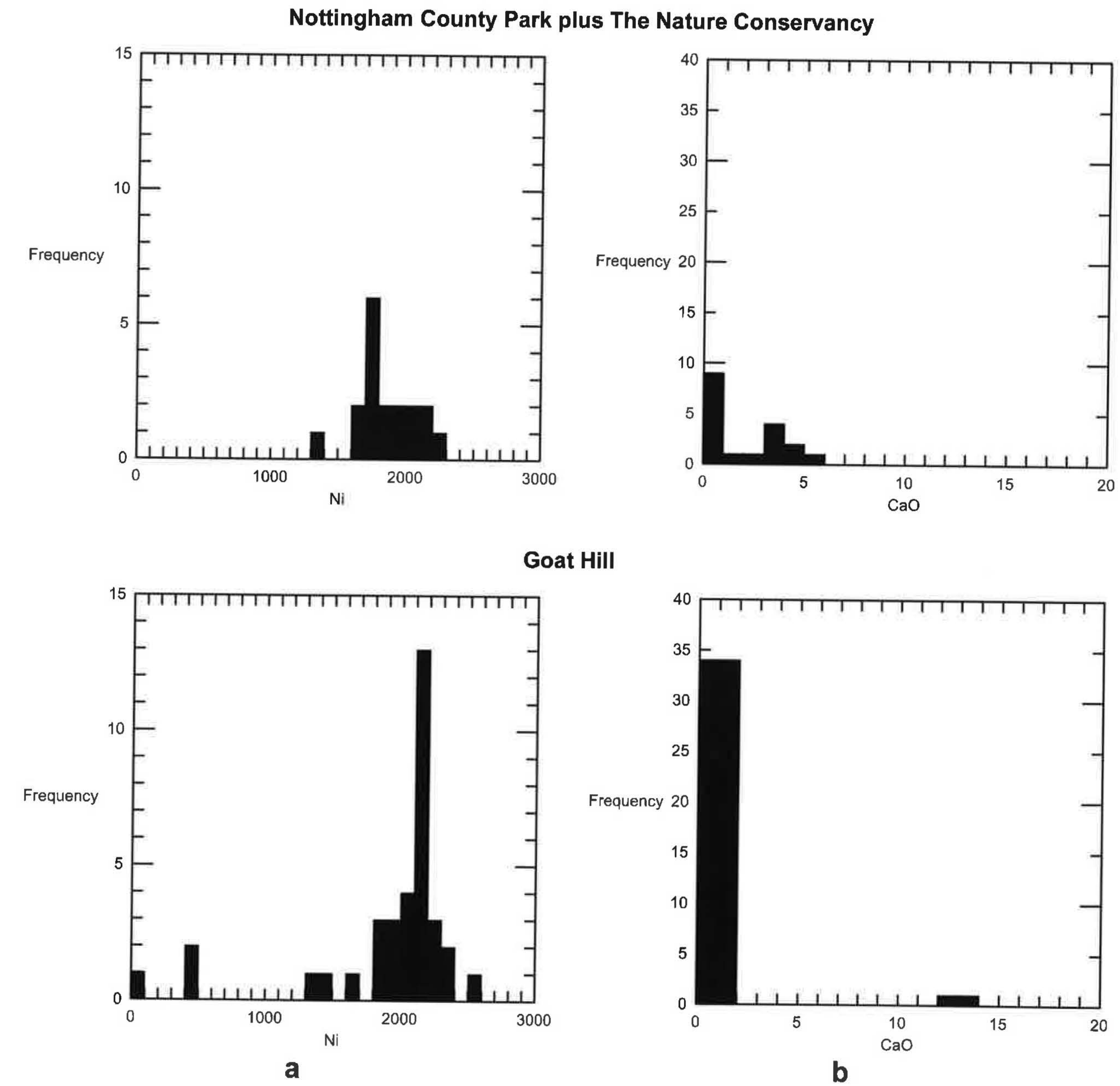

Figure 4. a. Histograms for Ni, a strong component of the serpentine factor, in composite bedrock samples from Goat Hill and, for comparison, Nottingham County Park plus the adjacent tract to the northeast owned by The Nature Conservancy. The highest Ni concentrations should better support serpentine endemic-flora. b. Histograms for CaO, which ameliorates the serpentine factor. Higher CaO concentrations should suppress serpentine-endemic flora.

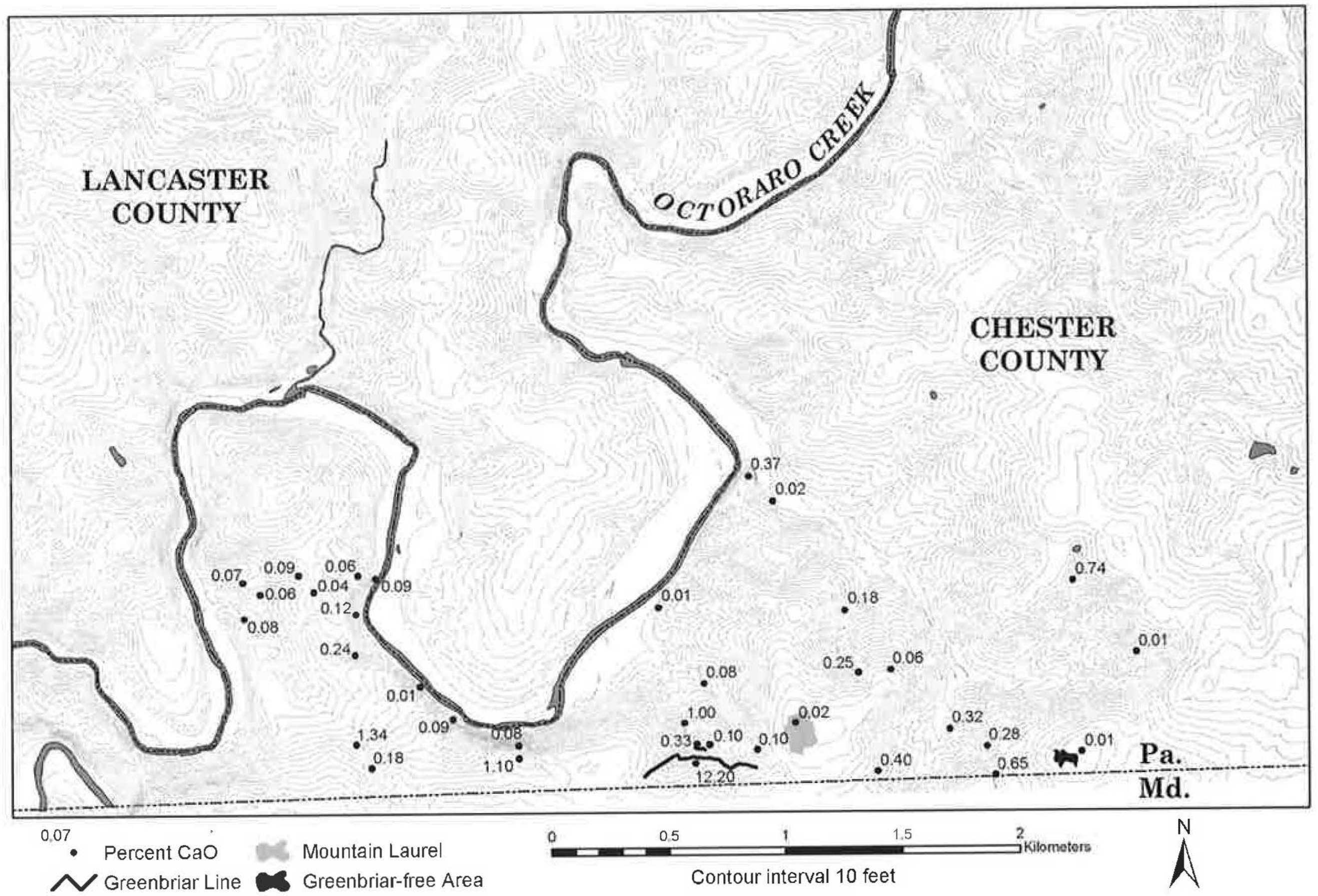

Figure 5. Plot of sample locations in the Goat Hill tract showing the concentration of CaO for each sample. For serpentine-endemic flora, it is desirable that the CaO concentration be as low as possible, preferably equal to or less than 0.1 percent.

groundwater from its source and then make acid where ferrous iron-bearing seeps are oxidized.) Not uncommonly, such yellow boy matures to cement alluvial sand and gravel to yield a "ferrcrete." These, too, are important habitats and should be protected from bursts of forest-fire-derived alkalinity, such as from potash lye, KOH. Presently these areas support high-bush blueberry (*Vaccinium corymbosum*) and sphagnum moss as well as protected species. Because some reactions that take place during the alteration of olivine to form serpentine (a process called serpentinization) are strongly exothermic (Evans 2004, p. 17), an abnormally warm microenvironment at the mouths of the acid seeps cannot be ruled out. If judicious canopy thinning were undertaken along a few areas of Pine Run, recolonization by *Drosera* and possibly even *Sarracenia* seems possible.

Observed non-anthropogenic floral effects

Figure 1 shows three areas where bedrock geology appears to be the primary factor affecting the flora, and for which no amount of management is likely to result in good habitat for serpentine-endemic flora. The first of these is bounded on the north by a roughly east-west "greenbrier line" marking the southern limit of luxuriant *Smilax* growth. Likely, it represents the upper (southern) contact of the serpentinite with the overlying gabbro which is richer in CaO, K_2O, P_2O_5, and lower in MgO and Ni. Management goals for the area south of the line should not be directly related to the serpentine environment.

The polygon shown nearly on the crest of Goat Hill itself (Figure 1) was created by determining the outermost locations of mountain laurel (*Kalmia latifolia*) growing on the hill. Here, the laurel has "found" an area that is still underlain by olivine, but because of elevation and time, it has been partly leached. This leaching may have been intense during the many extended, global-scale wet periods that have recurred in the past 58 million years (Bikerman et al., 1999). However, leaching is likely to be continuing via the following reaction that is largely completed in place:

$$Fe_2SiO_4 \text{ (fayalite, a form of olivine)} + H_2O = Fe_2O_3 + SiO_2 + 2H^+.$$

Evidence for such a reaction consists of the abundance of acid-loving flora, hematite (Fe_2O_3)-red soils, and thoroughly silicified serpentine and drusy quartz in the area. The resulting acidic, high ferric-iron-siliceous soils support mountain laurel and related flora, such as low-bush blueberry (*Vaccinium* sp.), and are quite distinct from normal serpentine soils which are typically nearly neutral. Interestingly, this area does not support serpentine flora despite the apparent retention of much of the Ni, which might be unavailable because of its strong adsorption on ferric oxides. The best use of the *Kalmia* polygon may be as a natural laboratory to help refine the proposed quantified serpentine factor. Otherwise, management goals for the area of this polygon and a buffer should not be directly related to serpentinite. Being as the leaching of olivine is likely still occurring, although at a reduced rate, and possibly providing beneficial Mg^{2+} to downslope seeps, protection from major disruption is still encouraged.

The anomalous, non-serpentine soils in the "greenbrier-free" area, shown in the extreme southeastern portion of the Goat Hill tract on Figure 1, appear to have formed by processes similar to those on the crest of Goat Hill. That is, the fayalite end member of previously unserpentinized olivine is currently being oxidized to yield acid, hematite, and quartz. This polygon was created by determining the location of greenbrier-free areas. Possibly, the soils are not as leached as those on the crest of Goat Hill, but ferric iron and silica are abundant and, for management purposes, this area and the *Kalmia* polygon are similar.

Another area believed to be underlain by serpentinite, but which is inaccessible to serpentine flora, is the large floodplain terrace of Octoraro Creek located northwest of Goat Hill. It, too, should not be managed for serpentinite flora but, if flooding considerations permit, might serve as an acceptable repository for any fertile soils that might be removed to expose fresh serpentinite habitat.

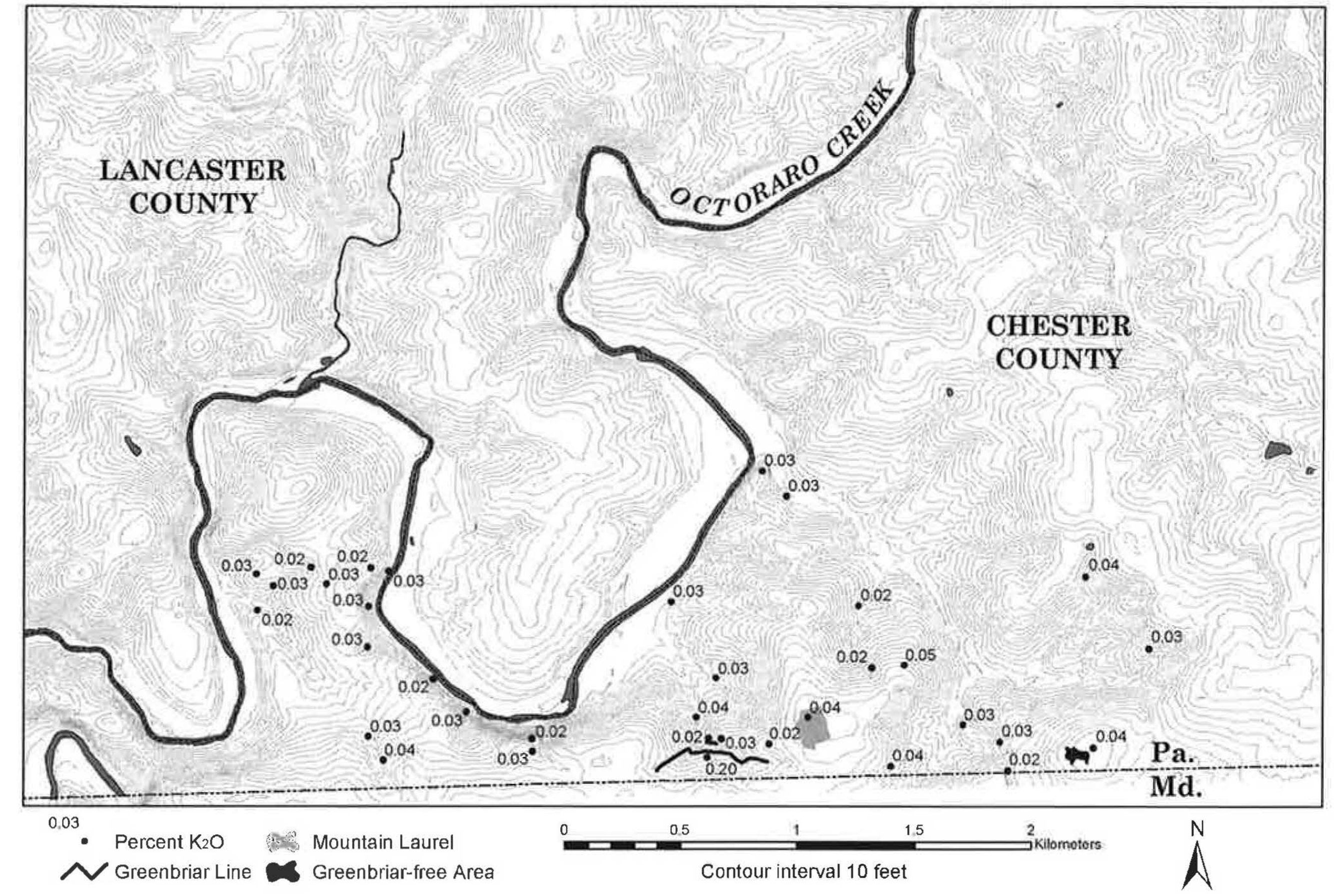

Figure 6. Plot of sample locations in the Goat Hill tract showing the concentration of K_2O for each sample. For serpentine-endemic flora, it is desirable that the K_2O concentration be as low as possible, preferably equal to or less than 0.03 percent.

Analytical results

Locations for the bedrock composite samples are presented in Table 1 and values for major, minor, and selected trace-element analyses in Table 2. Interpretation of these data, as they pertain to the protection of serpentine habitat, depends on the position of the sampled location as well as the abundances of analyzed elements. In part, this is because of the importance of drainage and sun angle. Figure 1 shows the sample locations, and Figures 2, 3, 5, 6, and 7 show the content of the oxides and elements in the proposed quantified serpentine factor: MgO, Ni, CaO, K_2O, and P_2O_5. Figure 8 shows the bedrock distribution of the inert element chromium.

The bedrock content of MgO, Figure 2, is believed to be one of the major positive components of the quantified serpentine factor. High available MgO is believed to support endemic serpentine flora. Areas having the highest MgO should be considered as candidates for plots to test this assumption. As shown in Figure 2, many areas in the Goat Hill tract having >38 percent MgO in the bedrock are available and this suggests the potential for excellent habitat.

The bedrock content of Ni, Figure 3, is believed to be a strong component, per unit concentration, of the serpentine factor. Areas having the highest available Ni should be considered as candidates for test plots. Many areas in the Goat Hill Tract appear to be underlain by bedrock containing >2100 ppm (0.21 percent) Ni. As shown in the histograms in Figure 4, such areas are less common in Nottingham County Park. The availability of Ni to flora depends on the host minerals as well as the total amount present. The availability likely decreases in the following order: Ni sulfides or arsenides > Ni substituting for Mg in forsterite > Ni in other silicates such as serpentinite > Ni adsorbed on iron oxides. Thus, the Ni in the area of insoluble residue sample G20 is not available to the flora. Research on the biological availability of Ni might be considered as a future refinement of the proposed quantified serpentine factor. Pennington (1973) provided a good start with data on the distribution of Ni in different soil fractions near the Red Pit chrome mine, Fulton Township, Lancaster County. Until such research, it can be reasonably assumed that the Ni remaining in the iron-rich insoluble residues on the hillcrests is probably not readily available to the flora.

The bedrock content of CaO, Figure 5, is a strong negative component of the serpentine factor. CaO is the active component of the different forms of agricultural "lime." Lime is well known to ameliorate the serpentine factor, enabling even many commercial crops to grow on serpentine after its application. Test plots should be considered for areas having the lowest possible CaO, preferably <0.1 percent. Fortunately, much of the bedrock in the Goat Hill tract contains less than this amount and, as illustrated in Figure 4, this is very favorable compared to Nottingham County Park, where most values exceed 0.1 percent CaO.

K_2O, also known as potash, the bedrock content of which is shown in Figure 6, is one of the three major plant nutrients. Potash is normally present in bedrock in amounts up into the percent range. Normal soils contain abundant available potash. Unless fertilized intentionally or otherwise, soils on serpentinites are typically very low in potash. In this case, the tree canopy is the major reservoir of soluble potash which is only slowly made available during normal decay or all at once in the case of a hard burn. Fortunately, the natural levels of total potash at Goat Hill are low. Potash levels as

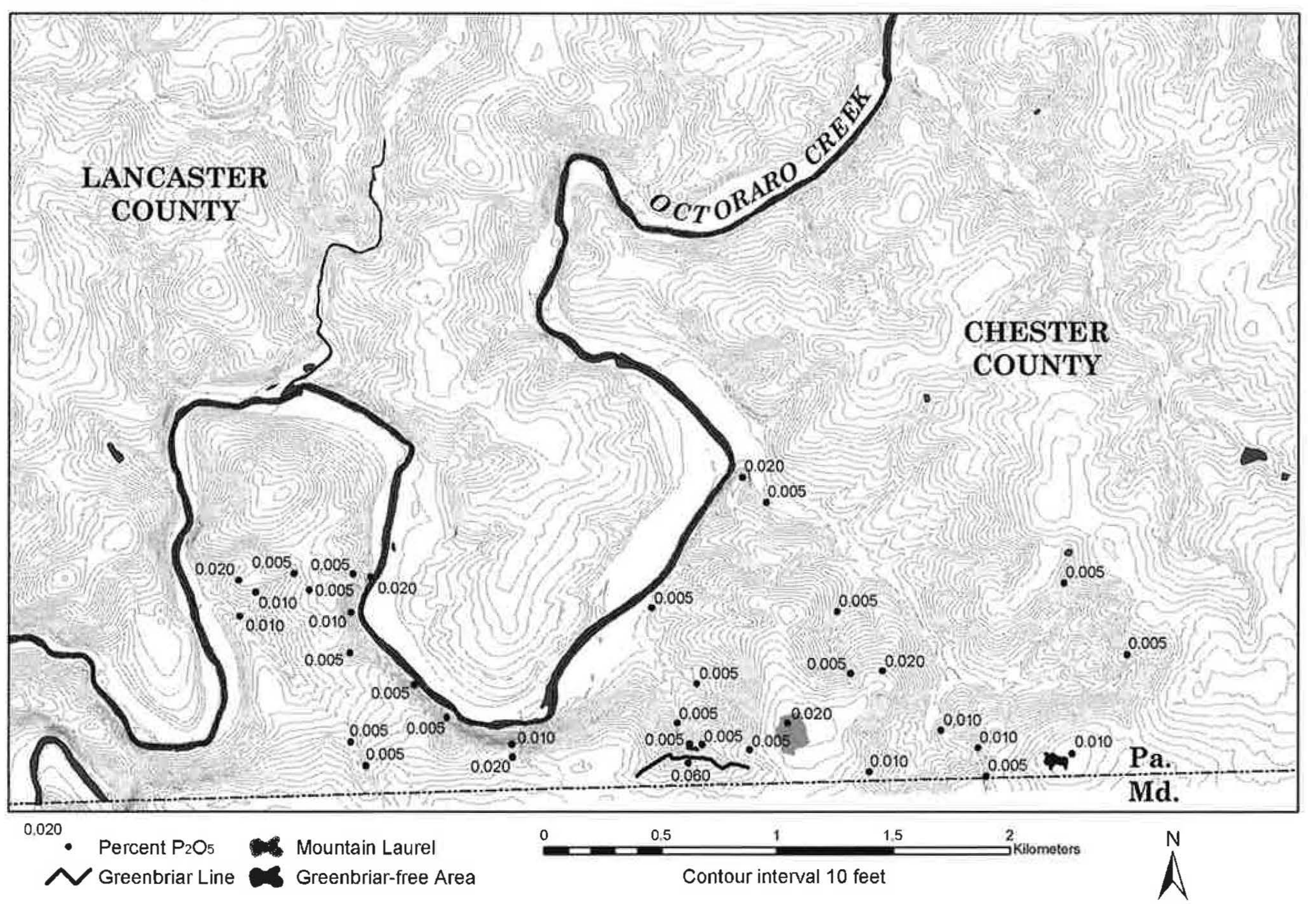

Figure 7. Plot of sample locations in the Goat Hill tract showing the concentration of P_2O_5 for each sample. For serpentine-endemic flora, it is desirable that the P_2O_5 concentration be as low as possible, preferably equal to or less than 0.005 percent.

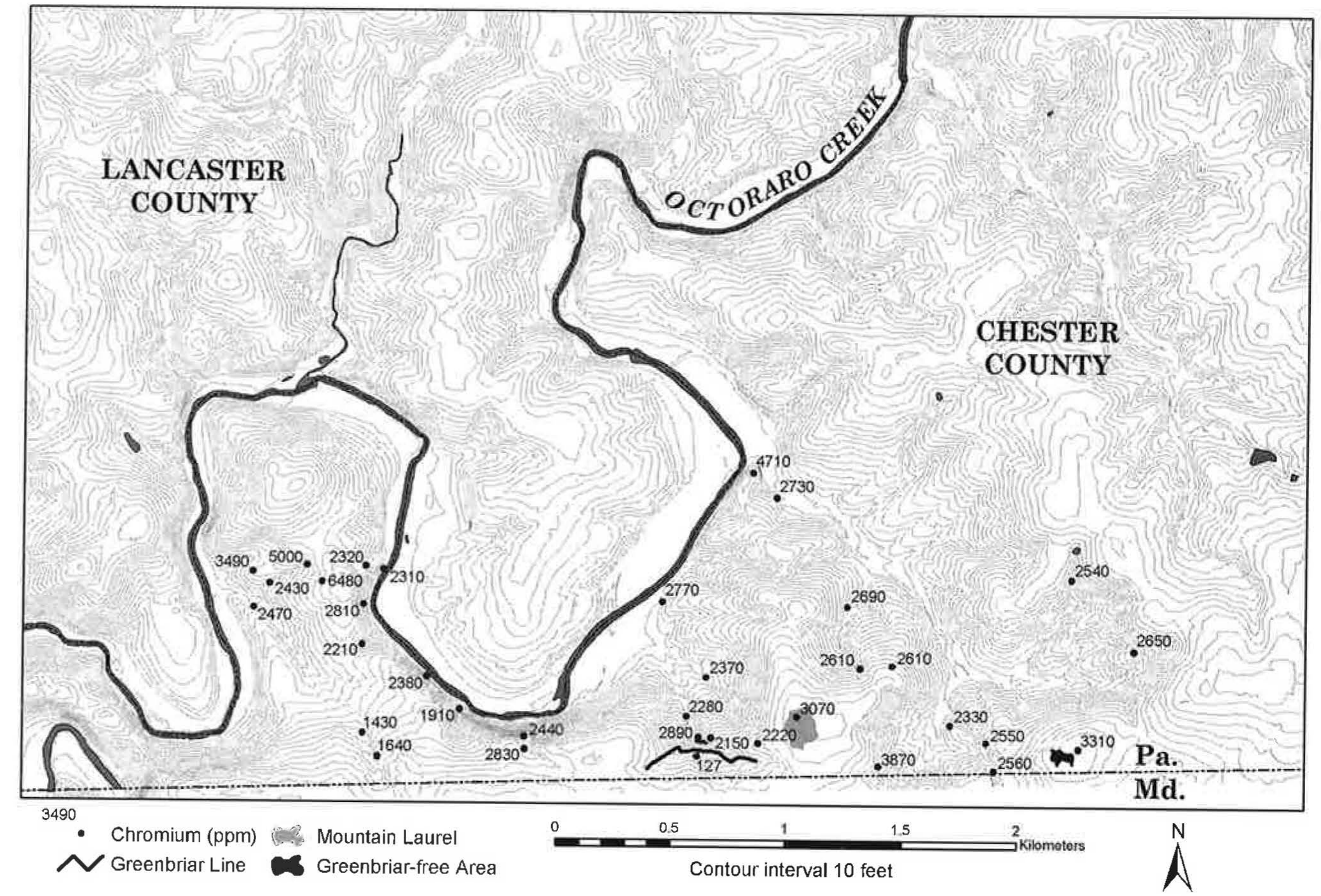

Figure 8. Plot of sample locations in the Goat Hill tract showing the concentration of chromium for each sample. Although chromium is not part of the serpentine factor, it is desirable that the chromium concentration be as high as possible.

low as possible are desirable and test plots might be restricted to areas having <0.03 percent potash. Timber removal in those areas would help insure continued low potash availability as might game management.

P_2O_5, the serpentine bedrock content of which is shown in Figure 7, is also one of three major plant nutrients. P_2O_5 is present in normal bedrock at the low 0.N percent levels, but it is at 0.00N percent levels in serpentine. As a result, unless fertilized intentionally or otherwise, soils on serpentinites are typically highly deficient in P_2O_5. P_2O_5 levels as low as possible are desirable, and in the Goat Hill tract, levels <0.005 are desirable for test plots. Potash management practices should generally suppress P_2O_5 as well.

Cr, the bedrock content of which is shown in Figure 8, is a good indicator of how mafic a rock is and therefore a good check on assumptions based on MgO and Ni, even though Cr is typically much too insoluble to be a part of the quantified serpentine factor itself. The correlation of Cr with MgO and Ni at Goat Hill is good and supports this thesis. Also, the primary carrier of Cr, chromite-group minerals, are field-visible. Their relative abundance in rock or accumulation in small natural riffles as black sand should provide useful field criteria for extrapolation between laboratory-analyzed bedrock composites. Rock dumps from chromite mining, such as near G4, -6, -33, and -34, are likely a high-unit-value habitat and potentially worthy of hand-thinning the *Smilax* and tree canopy.

Combined serpentine factor

Intuitive combination of the factors believed to be incorporated into the serpentine factor and likely, unconscious biases from reading studies summarized by Robert R. Brooks (1987), have led to the following proposed formula:

$$\text{Serpentine Factor} = (MgO + 50\,Ni) / (10\,K_2O + 10\,P_2O_5 + CaO)$$

If this ultimately proves not to be the best formula for use in the State Line District, the raw bedrock composition data provided in Table 2 will permit future iterations.

Data for the quantitative serpentine factor for the Goat Hill tract have been plotted geographically in Figure 9. It is proposed herein that the highest values might provide the best habitat for serpentine endemics. Those areas having a quantified serpentine factor value >100 are believed to be worthy sites for test plots if they are on narrow, well-drained ridges or on south-facing slopes that are likely to dry thoroughly. Though they might support other interesting habitat, areas having serpentine factor values <60 are believed to be unsuitable for most serpentine endemics. Also, steep, north-facing slopes may be receiving Ca from seeps and support

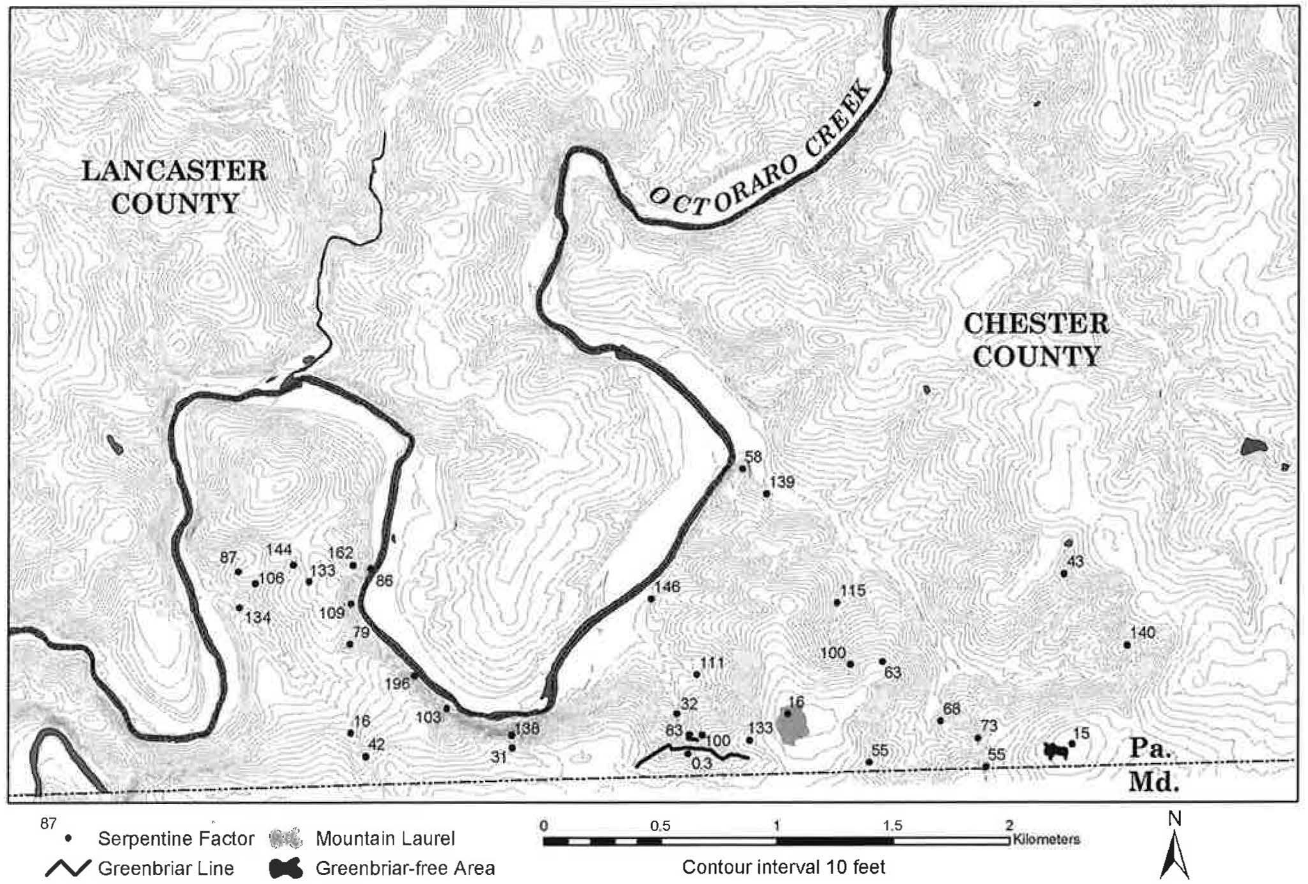

Figure 9. Plot of sample locations in the Goat Hill tract showing the serpentine factor for each sample. Serpentine factors greater than 100 are the most desirable and those less than 60 are unsuitable for serpentine-endemic flora.

other interesting flora. The area of G23 (Figure 1) is one such example.

Three test areas are recommended for consideration of timber and soil removal in Phase 1. During Phase 1, areas as small as 1 acre might be considered for treatment and evaluation. One recommendation each has been made for the western, central, and eastern portions of the Goat Hill tract:

1) The area east of G34 overlooking Octoraro Creek.

2) The crest of the east-west trending rib to the east of sample G35.

3) The already somewhat cleared strip to the southwest of G29.

If, after a few years of monitoring by botanists and other scientists, appropriate flora appear to be naturalizing these areas, they might be expanded to larger areas covering much of the crests. At that time, three additional 1-acre test plots might also be established:

4) On the south-facing slope of the area between G9 and G33.

5) On the south-facing slope of the rib between G32 and G19.

6) Possibly on the narrower portion of the east-west rib about halfway between samples G35 and G1, but where adequate bedrock for sampling was not found during the present study.

If such areas are cleared, it should be possible for a trained geologist/geochemist to obtain bedrock composite samples using the same methods as used in this study. If these were to be analyzed chemically and the flora of the cleared areas quantitatively evaluated after a decade or so, it should be possible to propose a refined quantitative serpentine factor using discriminant analysis and possibly correlation coefficients. Likely, the results of such studies could be applied with success elsewhere in the State Line Serpentine District.

ACKNOWLEDGEMENTS

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RESEARCH NOTE:
OPTIMIZATION OF DNA ISOLATION FROM FEATHERS[1]

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ABSTRACT

In genetic analyses, genotyping errors occur with greater frequency in samples with low DNA content. DNA can be isolated from feathers, but the yield is typically lower than that of blood or other tissue samples. In this study, DNA was isolated from three types of feathers using two sample preparation methods for three isolation protocols. The goal of the study was to identify the protocol that would maximize both DNA yield and isolate purity. The isolation protocol that resulted in the greatest DNA yield and isolate purity varied with feather type. Researchers who wish to isolate DNA from feathers for molecular analysis should be aware that the optimal isolation protocol may be dictated by feather type. [J PA Acad Sci 82(1): 48–51, 2008]

INTRODUCTION

In molecular studies, researchers often choose among multiple sampling schemes designed to obtain DNA from an organism of interest. Sampling may range from extremely invasive procedures, which induce considerable stress, to noninvasive procedures, which do not stress sampled animals (Bello et al. 2001). Noninvasive techniques are generally preferred over invasive techniques, especially for rare species (Segelbacher 2002). Plucking head and breast contour feathers may be a less invasive procedure than drawing blood from birds. The use of non-invasive techniques may reduce the stress of birds captured and processed in the field. However, the amount of extractable DNA is lower in feathers than in blood or other tissue samples (Segelbacher 2002), and therefore it may be problematic in genetic analyses. Furthermore, the DNA content of feathers varies by feather size with smaller feathers containing less DNA (Bello et al. 2001).

It is not clear whether different sample preparation techniques affect DNA yield from feathers or whether DNA is more easily isolated from certain feather types. In this study, DNA was isolated from three feather types using three isolation protocols and two sample preparation methods to investigate which of these factors affected DNA yield. Isolate purity was also assessed because it is desirable to perform molecular analyses on isolates of the highest possible purity. The protein content of each sample was measured to quantify the purity of the isolates produced by each protocol. The method, the produced isolates with the greatest DNA yield and highest purity (lowest protein yield) deemed the optimal protocol for each feather type.

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METHODS

Experimental design

Genomic DNA was extracted from three feather types, head and breast contour feathers, rectrices (flight feathers of the wing), and remiges (feathers of the tail). Twelve samples were analyzed for each feather type. The experiment was replicated twice (n=48). Two sample preparation methods were used to process calami; half of the samples were minced with a scalpel, and half of the samples were frozen in liquid nitrogen and pulverized with a mortar and pestle. The experiment was performed using feathers from three passerine bird species, *Ammodramus savannarum* (Grasshopper Sparrow), *Hylocichla mustelina* (Wood Thrush), and *Passerina cyanea* (Indigo Bunting). These male birds were window-kills from Compton Science Center, Frostburg State University, Frostburg, MD, except for the male Grasshopper Sparrow, which was a mist-net fatality from Ammer (2003). Birds were stored less than one year at -20° C. No blood feathers or calami containing a blood clot in the superior umbilicus were used in the experiment. DNA was isolated from 4.5 ± 0.5 mg of tissue for each sample. The DNA and protein concentrations (µg/ml) of each isolate were quantified using the DNA analysis/concentration option on a Genesys™ 2 Spectrophotometer (Thermo-Spectronic, Rochester, NY). Isolates were diluted 1:10 with deionized water, and the absorbance was read as 260/280 nm. Total DNA and protein yields (µg) were calculated by multiplying the isolate concentration by the isolate volume and 10 (the dilution factor). Three DNA isolation protocols were tested, and each method was used on four replicate samples of each feather type.

DNeasy® isolation protocol

A DNeasy® Tissue Kit (QIAGEN Inc., Valencia, CA, USA) was used to isolate DNA from feathers. Methods were

modified from the DNeasy® Tissue Handbook (March 2004, Qiagen). Calami were removed, minced or pulverized in liquid nitrogen, and placed in a 1.5 ml microcentrifuge tube with 200 µl of Buffer X1 (lysis buffer 1) (10 mM Tris Cl; pH 8.0, 10 mM EDTA, 100 mM NaCl, 40 mM DTT, 2% SDS, and 250 µg/ml Proteinase K). Samples were incubated overnight at 55° C with agitation. After the incubation, 200 µl of Buffer AL (lysis buffer 2) was added, and samples were incubated at 70° C for 30 min. After the addition of 200 µl of 100% ethanol, samples were placed into DNeasy® mini spin columns in 2 ml collection tubes and centrifuged at 6000 × g for 1 min. Flow-through and collection tubes were discarded following centrifugation. Spin columns were washed by centrifugation with 500 µl Buffer AW1 (wash buffer 1) (6000 × g for 1 min) and then 500 µl Buffer AW2 (wash buffer 2) (25000 × g for 3 min). Flow-through and collection tubes were discarded after each wash. Forty µl of Buffer AE (elution buffer) was added to spin columns placed in 1.5 ml microcentrifuge tubes. Samples were incubated at room temperature for 1 min before being centrifuged at 6000 × g for 1 min. The flow-through was collected following centrifugation. The elution step was performed twice to yield a final isolate volume of 80 µl. Isolates were stored at 4° C.

InstaGene™ isolation protocol

The protocol was modified from Goossens et al. (1998). Calami that were either minced, or pulverized in liquid nitrogen and placed in a 1.5 ml microcentrifuge tube with 200 µl of InstaGene™ matrix (Bio Rad), 250 µg/ml Proteinase K, and 40 mM DTT (lysis/purification buffer). Samples were incubated overnight at 65° C with agitation and then centrifuged at 9300 × g for 30 sec. The samples were incubated in a heat block at 110° C for 10 min and then centrifuged at 13200 × g for 3 min. The supernatant was removed and centrifuged at 13200 × g for 3 min to ensure that all remaining feather tissue and InstaGene™ matrix beads were removed. Isolates were stored at 4° C.

Phenol/chloroform isolation protocol

The protocol was modified from Taggart et al. (1992). Calami that were either minced, or pulverized in liquid nitrogen and placed in a 1.5 ml microcentrifuge tube with 500 µl of lysis buffer (50 mM Tris; 0.1 M EDTA, 1% SDS, 0.4 mg/ml Proteinase K, and 40 mM DTT, pH=8.0). Samples were incubated overnight 37° C with agitation, and then equal volumes of phenol were added. Samples were centrifuged at 15400 × g for 20 min, and the supernatant was removed and transferred to a clean microcentrifuge tube. An equal volume of phenol/chloroform (1:1) was added, and the phases separated by centrifugation at 15400 × g for 5 min. The supernatant was removed and transferred to a clean tube. An equal volume of chloroform was added, and the phases were separated by centrifugation at 15400 × g for 5 min. The supernatant was removed and transferred to a clean tube, and 500 µl of cold 95% ethanol was added to the samples until a precipitate was seen. Samples were placed at -80° C for 1 hr, and then the precipitate collected by centrifugation at 13000 rpm for 30 min. Supernatants were removed, and samples were dried in a fume hood. Pellets were resuspended in 80 µl of nuclease free water and stored at 4° C.

Statistical analysis

A 3 × 3 × 2 factorial ANOVA (Zar 1999) was used to test for differences in DNA and protein yield among the means of replicate samples and interaction between factors. The three factors were the isolation protocol, feather type, and sample preparation method. There were three levels of isolation protocol (DNeasy®, InstaGene™, and phenol/chloroform) and three levels of feather type (contours, rectrices, and remiges). There were two levels of sample preparation method (minced and liquid nitrogen). The least significant difference test (Zar 1999) was used for all multiple comparisons. This post-test was chosen because of its consistency at detecting differences regardless of the size and quantity of means in the experiment. All analyses were conducted using the SAS software package (SAS Institute Inc. 1999).

RESULTS

DNA yield

The isolation protocol that resulted in the greatest DNA yield varied with feather type. A statistically significant interaction was observed between the isolation protocol and feather type factors ($F = 10.60$, df = 4, $P < 0.001$). The InstaGene™ isolation protocol was the most effective method for isolating DNA from contour feathers with a mean yield of approximately 50 µg (Table 1). This treatment combination produced the greatest DNA yield among all combinations. The phenol/chloroform isolation protocol was the most effective method for isolating DNA from rectrices and remiges with mean yields of approximately 44 µg. The DNeasy® isolation protocol was the least effective method for isolating DNA from all feather types with mean yields from the three treatment combinations ranging from approximately one to two micrograms. The sample preparation method did not affect DNA yield as both the three-way interaction among all factors ($F = 1.02$, df = 4, $P = 0.422$) and single factor ($F = 0.52$, df = 1, $P = 0.481$) were not statistically significant to the model.

Isolate purity

The isolation protocol that resulted in the greatest isolate purity (lowest protein yield) also varied with feather type. The DNeasy® isolation protocol was the most effective

Table 1. Mean yield (μg) ± SE of DNA and protein from 4.5 mg of tissue from three feather types using three isolation protocols and two sample preparation methods. Mean ± SE DNA Purity ($A_{260\ nm}/A_{280\ nm}$) for each feather type, isolation protocol, and sample preparation method.

Protocol	Feather Type	Mean ± SE DNA Yield (μg) Sample Preparation Method		Mean ± SE Protein Yield (μg) Sample Preparation Method		Mean ± SE DNA Purity ($A_{260\ nm}/A_{280\ nm}$) Sample Preparation Method	
		Minced (n=2)	Pulverized (n=2)	Minced (n=2)	Pulverized (n=2)	Minced (n=2)	Pulverized (n=2)
DNeasy®	Contour	0.83 ± 0.46	3.30 ± 3.90	0.34 ± 0.19	23.31 ± 22.01	2.000 ± 0.00	1.393 ± 0.20
	Rectrices	1.74 ± 1.24	0.78 ± 0.20	3.31 ± 4.69	8.48 ± 0.08	1.765 ± 0.41	1.343 ± 0.10
	Remiges	1.80 ± 0.13	1.26 ± 1.07	6.04 ± 8.54	0.52 ± 0.44	1.823 ± 0.40	2.000 ± 0.00
InstaGene™	Contour	50.57 ± 5.60	51.59 ± 0.35	641.58 ± 60.56	1042 ± 220.91	1.325 ± 0.04	1.128 ± 0.07
	Rectrices	21.18 ± 6.09	25.76 ± 8.94	2516.28 ± 11.77	2424 ± 203.22	0.707 ± 0.04	0.737 ± 0.04
	Remiges	28.39 ± 1.84	40.89 ± 0.55	2632.80 ± 477.20	1658 ± 303.06	0.745 ± 0.04	0.914 ± 0.05
Phenol/chloroform	Contour	35.05 ± 7.69	29.47 ± 9.41	879.72 ± 243.64	679.20 ± 288.50	1.053 ± 0.02	1.086 ± 0.04
	Rectrices	44.35 ± 7.24	39.79 ± 11.69	923.20 ± 70.14	937.16 ± 289.12	1.113 ± 0.09	1.072 ± 0.01
	Remiges	46.50 ± 11.31	38.27 ± 13.89	2632.80 ± 477.20	1658 ± 303.06	1.032 ± 0.02	1.142 ± 0.09

method for maximizing the purity of isolates for all feather types with mean protein yields from the three treatment combinations (Table 1). The phenol/chloroform isolation protocol produced isolates with intermediate levels of purity (mean yields of approximately 1000 μg of protein) when used on all three feather types, as did the InstaGene™ protocol when used on contour feathers. The InstaGene™ isolation protocol resulted in the isolates of lowest purity when used on rectrices and remiges with mean protein yields over 2000 μg. The InstaGene™ isolation protocol used on rectrices produced isolates with the lowest purity among all treatment combinations with a mean protein yield of approximately 2500 μg (Table 1).

Isolate purity was also affected by different combinations of feather types and sample preparation methods. A statistically significant interaction was observed between the sample preparation method and feather type factors ($F = 6.77$, df $= 2$, $P = 0.007$), although the three-way interaction among all factors was not statistically significant to the model ($F = 2.55$, df $= 4$, $P = 0.080$). However, the least significant difference post-test failed to detect significant differences between the means of any of the treatment combinations. By examining the means, it appeared that the interaction of sample preparation method and feather type was mostly the result of differential protein yields between remiges processed by mincing and liquid nitrogen pulverization. Remiges that were minced produced the DNA isolates of lowest purity with mean protein yields of approximately 1500 μg. In contrast, the remiges processed by liquid nitrogen pulverization resulted in DNA isolates with mean protein yields of approximately 1000 μg. Both sample preparation methods produced DNA isolates with mean protein yields of approximately 1000 μg when used on rectrices. Both sample preparation methods also resulted in isolates of similar purity (mean yields of approximately 500 μg of protein) when used on contour feathers. These two treatment combinations produced the isolates of greatest purity among all treatment combinations.

DISCUSSION

DNA yield

Researchers who wish to isolate DNA from feathers should be aware that different isolation protocols may be more or less effective at isolating DNA, depending on the type of feather used. The results of this study suggest that the InstaGene™ isolation protocol should be used to isolate DNA from contour feathers. The phenol/chloroform protocol should be used when isolating DNA from remiges or rectrices. Because only three isolation protocols were tested in this study, direct comparisons of additional protocols may reveal other methods that are more efficient at isolating DNA from these feather types.

The results of this study indicate that the DNeasy® isolation protocol should not be used to isolate DNA from feathers. The low DNA yields obtained with this protocol may have been caused by the silica gel membranes in the mini spin columns. During the procedure DNA was bound to silica gel membranes, the membranes were washed to remove impurities, and the DNA was eluted from the membranes. There were two likely points at which DNA may have been lost. Either DNA was stripped from the membranes and discarded with the filtrate during the wash steps, or DNA remained bound to the membranes after elution. The other two isolation protocols did not use silica gel membranes to purify DNA, and they did not result in the strikingly low yields that the DNeasy® isolation protocol produced. Alternately, low yields associated with the DNeasy® isolation protocol may have been attributed to poor performance of the lysis buffer used with this procedure. Keratin, a main component of the calamus, is a difficult protein to break down. DNA may have remained inside pieces of undigested calami if the buffer did not adequately denature keratin, resulting in reduced yields. However, because the lysis buffers used in each of the isolation protocols were similar in chemical composition, low DNA yield in the DNeasy® isolates was unlikely the result of incomplete keratinolysis.

In studies where molecular analyses will be performed on DNA isolated from samples with low DNA content (e.g. feathers or hair), it is recommended that researchers run optimization experiments to find the isolation protocol that will maximize DNA yields. Performing such experiments could directly increase the quality of the study if the number of samples with low quantities of DNA is reduced. Genotyping and haplotyping errors may occur with greater frequency when amplifying samples with low DNA quantities (Goosens et al. 1998, Sefc et al. 2003, Segelbacher 2002). Additionally, amplification may fail entirely if DNA concentrations of samples are low (Sefc et al. 2003, Segelbacher 2002). Using a protocol that maximizes DNA yield may help reduce errors and increase sample sizes in molecular studies if the DNA content of isolates is increased.

Isolate purity

Researchers who wish to isolate DNA from feathers should also be aware that different isolation protocols may yield isolates of varying purity, according to the feather type used. Although protein yield was affected by feather type, the DNeasy® isolation protocol produced the isolates of highest purity, regardless of feather type. However, this isolation protocol also resulted in isolates with the lowest DNA content. It appears that the silica gel membrane system of the DNeasy® protocol was both beneficial to isolate purity and detrimental to DNA yield. Ideally, the optimal isolation protocol should maximize both DNA yield and isolate purity. However, as the results of this study suggest, this is not always possible. Instead, researchers should examine the advantages of different protocols. Maximizing DNA yield should be the primary concern when choosing the optimal protocol. Isolate purity should be a secondary consideration because there are procedures to further purify isolates after DNA has been isolated. The results of this study suggest that the phenol/chloroform isolation protocol should be used on rectrices and remiges, and the InstaGene™ protocol should be used on contour feathers. These combinations of isolation protocols and feather types resulted in the best compromise between DNA content and purity of the isolates.

Further study is needed to fully understand how the interaction betwen sample preparation method and feather type affects isolate purity. The results of this study revealed that remiges processed by liquid nitrogen pulverization produced isolates of greater purity than those produced by mincing remiges. Perhaps the liquid nitrogen pulverization created more surface area than the mincing method, leading to more efficient digestion of the samples by proteolytic enzymes. However, it is suspicious that this was the only treatment combination that displayed a differential affect between sample preparation methods. Furthermore, similar results would have been expected for both remiges and rectrices because the calami of these feathers were similar in size. It is possible that the interaction between sample preparation method and feather type was a spurious result. The fact that the experiment was performed with only two replications supports this hypothesis. Unfortunately, the amount of replications was limited by the availability of feathers. Future optimization experiments should be replicated three or more times. The isolation protocol that maximizes DNA yield may not produce isolates of the highest purity. Optimization experiments may be used to increase the quality of molecular studies by identifying the most efficient DNA isolation procedure.

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